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CONTENTS

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SEX STUDIES

X. THE CORPUS LUTEUM IN THE OVARY OF THE DOMESTIC FOWL[1]

RAYMOND PEARL AND ALICE M. BORING

SIX TEXT FIGURES AND NINE PLATES

I. INTRODUCTION

The corpus luteum is one of the clearly recognized sources of an internal secretion in the mammal. Various functions have been ascribed to it. Its function in connection with secondary sex characters has been discussed by Pearl and Surface ('15), with one piece of clear cut evidence. The case was that of a cow which developed cystic ovaries and took on male secondary sex characters. The ovaries were compared histologically with those of a normal cow and the two were found to resemble each other in all respects except that the cystic ovaries had no corpora lutea. The interstitial cells were the same in both so that the difference in secondary sex characters could not be attributed to them. The implication of the facts is that the corpus luteum has an inhibitory influence in the female which prevents maleness from developing and that when no corpus luteum is formed, male characters appear.

The chief difficulty with such a view has been that its application is very limited, as the corpus luteum has been supposed to be a structure occurring only among mammals. The secondary sex characters of birds are particularly pronounced and the results of ovariotomy experiments, such as those of Goodale, ('16) show the possibility of changing these characters experimentally. Also the many cases of hermaphrodite birds (to be

[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 115.

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considered in Study XI of this series), with varying degrees of maleness and femaleness indicate the presence of some sex regulating substance in birds. Is this substance entirely different from the corpus luteum probably connected with it in mammals, or is there a corpus luteum or its homologue in birds? An investigation of this question has been undertaken in this study. We consider that we have successfully demonstrated the presence of the corpus luteum in the domestic chicken. Further discussion of the bearing of this fact on the whole question of secondary sex characters will be deferred until a later paper of this series, which will unfortunately probably be delayed for some time, as one of the authors (R. P.) has been called upon by the government to turn his attention to practical problems during the war.

A careful examination of the ovary of a bird which has been actively laying shows three kinds of structures: the yolks of various sizes indicating different stages of development, the discharged follicles in various stages of regression, and the atretic follicles or degenerating eggs of different sizes. These are all easy to identify when they are large enough to protrude far from the surface of the ovary, that is, when they are larger than 2 or 3 mm. in diameter. Under this size, it is impossible to distinguish the discharged follicle from the atretic. Both of them show a yellow or orange spot in the center. The question naturally arises whether these yellow spots are homologous in structure and origin with the mammalian corpus luteum. They never develop into a large mass like the corpus luteum of the mammal. They have the color of the spots on the cow ovary which indicate remains of old corpora lutea. In order to interpret these yellow spots, a study has been undertaken of the progressive and regressive changes in the cell structure of egg follicles in different conditions, undischarged, discharged and atretic.

The material used came chiefly from four birds, an actively laying Bantam, a Barred Plymouth Rock in the same condition, an old Compine past the laying condition, and a guinea-hen with a large ovary containing several large yolks. Material

from a number of other birds was used in the study of special points. These are some of the same birds used in Study IX. The ovaries were fixed in Gilson and McClendon. In the Barred Plymouth Rock ovary the different discharged follicles were sectioned separately and arranged in a series, according to size and consequent order of age since ovulation. After the study of this series, it was easy to judge of the condition of various follicles in pieces of the other ovaries cut at random. Various stains were tried, iron haematoxylin and Delafield's haematoxylin for general histology and Mallory's and Mann's stains for secretion granule tests.

II. UNDISCHARGED FOLLICLES OF THE HEN'S OVARY

A study of the follicles of large undischarged oocytes shows them to consist of an epithelial layer, the granulosa, and two connective tissue layers, the inner and the outer theca folliculi (fig. 1). In the inner theca are located groups or nests of epithelial cells (*l,* figs. 1 and 2). They have been described by many authors, notably Ganfini, Sonnenbrodt and Poll, but have been called interstitial cells. Poll calls them Kornzellen at first, describes their collection into the internal theca and then implies their function by saying that the biological rôle of the theca interna in the formation of the corpus luteum still needs to be worked out. That he also confuses them with interstitial cells is shown by his statement that the theca interna fills up the atretic follicle with groups of Kornzellen, which is the same thing as an interstitial gland. These nests of cells in the bird are not anything like the usual glandular interstitial cells of the ovary in structure. They are about three times as large (compare fig. A and C). The nucleus is bigger and plumper, the cytoplasm is usually clear and vacuolated in appearance, only occasionally containing a few acidophile granules which stain with the fuchsin in Mallory's stain or the eosin in Mann's stain; while the real interstitial cells are crowded with granules. These large clear cells are seldom found alone, but are usually grouped into nests of various shapes, as already mentioned. The cytoplasm of these cells usually will not take

up an acid stain. They remain strikingly clear, when the connective tissue all around them is highly colored. So great is the contrast that they show distinctly even at low magnification in a section such as figure 1. Furthermore, they are found in

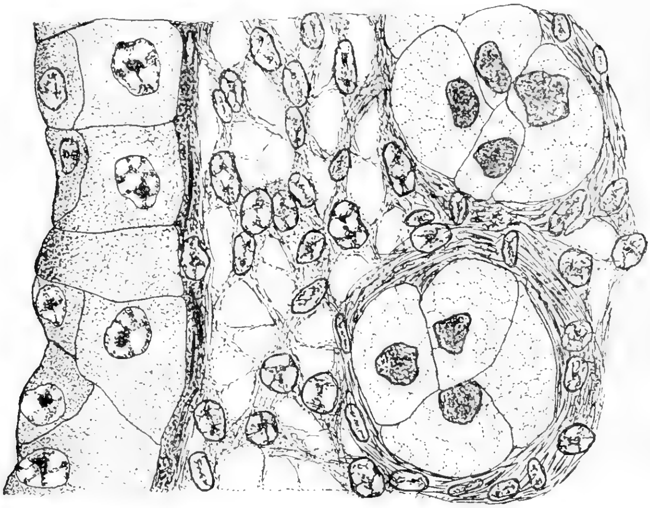

Fig. A Part of follicle of wall of medium sized oocyte in hen ovary. (× 950.) Compare figure 2.

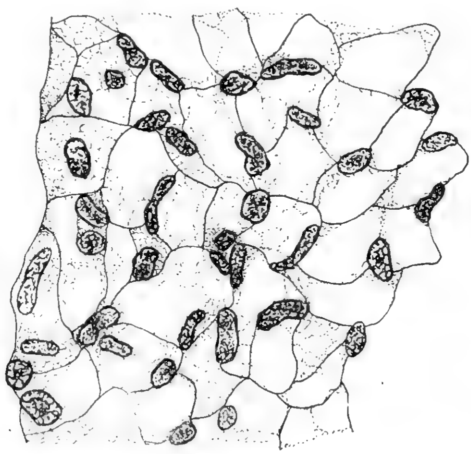

Fig. B Part of theca interna of sixth discharged follicle in hen ovary, showing many vacuolated lutear cells. (× 950.) Compare figure 6.

different parts of the ovary, mostly in the theca interna, while the interstitial cells lie in the general stroma, and especially on the periphery.

Figure 3 shows several very young oocytes from the same ovary as figure 1. In these, the follicle consists only of a single layer of epithelial or granulosa cells (*g*). The connective tissue layers are not yet formed. But there are nests of clear cells (*l*) in the stroma nearby. Presumably these are included with the connective tissue when the theca interna is formed.

III. DISCHARGED FOLLICLES OF THE HEN'S OVARY

In the largest follicles before ovulation, the three layers are stretched out very thin by the pressure of the large yolk within them. After ovulation, there is a shrinkage of the follicle walls, probably due to the elasticity of the connective tissue recoiling at the sudden release of pressure from inside. On the Barred Plymouth Rock ovary, the ripe yolk measured about 40 mm. in diameter, and the last discharged follicle measured 20 mm. in length from base to tip, while the next to last was 12 mm., and the fourth in the series was 7 mm. As this shrinkage in length takes place, the walls thicken until finally a small oval mass results having no resemblance to a hollow follicle. The ruptured place through which ovulation took place, becomes gradually closed up, by the growing together of the edges, and the filling of cells into the cavity. Sometimes this mass of cells protrudes slightly from the cavity at the old place of rupture, thus somewhat more resembling a miniature mammalian corpus luteum. Yellow pigment forms in the puckered edges of the follicle and also in the central mass.

The microscopic study of sections through discharged follicles of various ages shows that the increase of thickness of walls is due chiefly to a thickening of the theca interna. Figure 4 is a section of the last discharged follicle of the Barred Plymouth Rock ovary. It shows the thickened theca interna (*i*) and in addition the remnants of the granulosa (*g*). The latter seems to loosen from the follicle after ovulation, and the cells collect in masses in the cavity and degenerate.

The first subsequent discharged follicle in the series to show any new microscopic features is the sixth (fig. 5), where there appears a marked increase in the number of nests of vacuolated cells in the theca interna (*l*). They are concentrated toward the cavity. The closeness of nests together may be partly due to the shrinkage of the cavity after discharge of the egg. But as this does not seem sufficient to account entirely for the increase, the number must be added to either by division or migration. The fact that division plays some part in the process is proven by the observation of several mitotic spindles. The character of these cells shows better in greater magnification, as in figure 6 and figure B.

The further progress of the increase of vacuolated cells in the theca interna is shown in figure 7, a section of a discharged follicle too small to have been placed in the series as to time of discharge. Here the whole internal theca looks full of holes, due to vacuolated cells (*l*). The central cavity is nearly obliterated, almost as though the edges had been pulled up by a gathering string. There are, however, a few cells in the central cavity (*p*). These get in there by migration from the internal theca.

Figure 13 shows the process in an atretic follicle where it is more conspicuous, but it is true to a more limited extent in the discharged follicles. The cells concerned have a speckled appearance in figure 13 (*d*). They are abundant in the follicle wall, some are scattered among the yolk spheres in the central cavity and some are on the border line between the follicle wall and the cavity, indicating that the cells actually migrate into the cavity. Occasionally a very large central plug is formed which protrudes from the spot of rupture. Figure 7 shows a small plug of this kind (*p*).

The cavity usually becomes finally obliterated by the thickening of the internal theca and the formation of large masses of vacuolated cells from the original nests. In figure 8, the chief tissue consists of the masses in the internal theca (*l*). The line between the theca interna and externa is marked by the irregular spaces and blood vessels. The connective tissue in the

center (*c*) shows where the edges of the internal theca have drawn together and obliterated the cavity.

We have traced thus far the general histological changes involved in the shrinking and filling up of the discharged follicle. We must consider next in more detail, the cytology of these particular cells involved. Figure 2 and figure A show them in their original condition from a large undischarged follicle. We have earlier in this paper pointed out their especial characteristics in distinction to the interstitial cells. By the time they are close enough together to cause the vacuolated appearance of the whole inner part of the theca interna, the nuclei are somewhat shrunken and pushed to the side of the cell, suggesting active elaboration of secretion material (fig. 6 and fig. B). By the time the closing in of the follicle has neared completion (figs. 8 and 9), the character of the cells is decidedly modified (fig. C). The cell boundaries in any one small mass of cells are indistinguishable. The cells seem to have melted together so that the outlines of the vacuoles are the evidently visible lines rather than the cell outlines. The vacuoles also are much larger than previously. The nuclei are smaller and less regular in outline, they stain darker, in fact, they look shrunken. These figures show nicely the contrast between the cells which fill up this discharged follicle and the interstitial cells. The interstitial cells lie in the connective tissue of the external theca and of the internal theca in between the masses of transformed epithelial nest cells. They are entirely unchanged from their usual appearance. They show clearly because the granules with which they are packed stain vividly with acid stains. A homologous mass of cells from an older solidly filled follicle (fig. 10) is shown in figure 11 and figure D. Here the nuclei show still further signs of degeneration and the general network of the cytoplasm contains clumps that look like secretion material. These secretion particles are yellow in color. They look amorphous in character, and they vary greatly in size (fig. 20). They can not be fatty, for they have not dissolved in the clearing oils. They cannot be of the protein nature of the secretion granules of the interstitial cells, as they retain their distinct yellow color

no matter how the preparation may be stained. They make a fine contrast with iron haematoxylin, acid fuchsin, eosin, methyl blue, and still show their own characteristic yellow even with

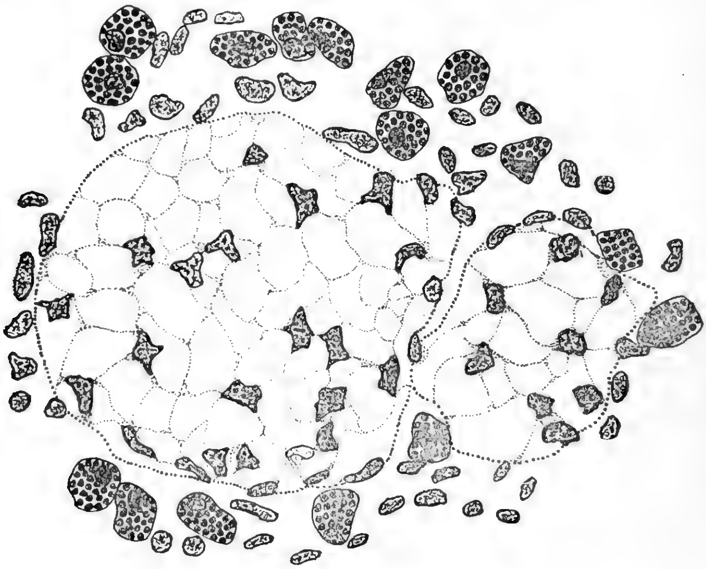

Fig. C Masses of lutear cells from older discharged follicle, with interstitial cells lying in connective tissue between masses. (× 950.) Compare figure 9.

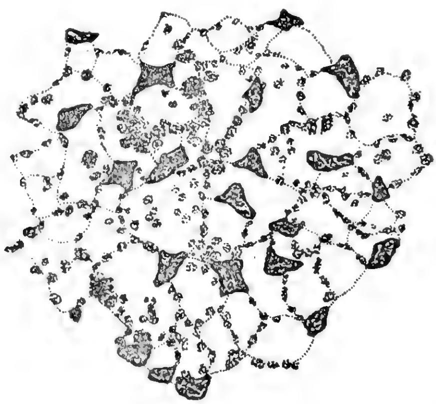

Fig. D Mass of lutear cells from discharged hen follicle, with pigment particles developed in the network. (× 950.) Compare figure 11 and figure 20.

orange G. The cell masses finally become nearly filled with this yellow material, some of it collecting in clumps several times larger than the degenerated nuclei.

Further tests of the character of the cell contents in these cell masses were made with Sudan III. Hand sections were made of material in McClendon's fluid. Although these could not be cut very thin, they showed that the inner lining of the early discharged follicles contains fatty material. In an old follicle with central yellow mass the cells of the yellow mass take the red of the Sudan III, but the yellow amorphous particles show in the midst of the red. They can be squeezed out of broken cells and isolated from the red fatty background, showing they are still yellow, unaffected by the Sudan III, and therefore not of a fatty nature. The fatty substance indicated by the Sudan III reaction in both young and old follicles is probably contained in the vacuoles so conspicuous in paraffin sections. The xylol would have dissolved out all the fat leaving the vacuoles in which it had been contained.

IV. DEGENERATION OF CORPUS LUTEUM IN COW OVARY

In order to show the significance of the yellow mass formed in the center of discharged follicles in the hen ovary, we have made a brief study of the degeneration of the corpus luteum in the cow ovary for comparison. There is an extensive literature on mammalian corpus luteum, but this deals chiefly with the development and early involution. Now the bird quite evidently has no structure similar to the large corpus luteum which fills up half the ovary of a cow at its full development. The small yellow spot on the bird ovary resembles the small yellow spots on the cow ovary which mark the old remains of former corpora lutea. Ovulation in the cow alternates between the two ovaries. So by studying the two largest corpora lutea on both ovaries we can arrange a series of four involution stages. Beyond that, they all seem equally shrunken and therefore can not be arranged in a further series. Such a series of four involution stages has been studied for two cows, and in addition several older corpus luteum remains.

The last formed corpus luteum is of a salmon pink color, due to a combination of the blood color and the lutein color. Sections show it composed of large plump cells with rounded nuclei, as described by Corner. These luteum cells are scattered in the midst of an areolar connective tissue groundwork (fig. 18 and fig. E). In dehydrating for embedding, the absolute alcohol and xylol become very yellow, indicating that the cells contain something soluble in these reagents. This is of course one chemical character of lutein.

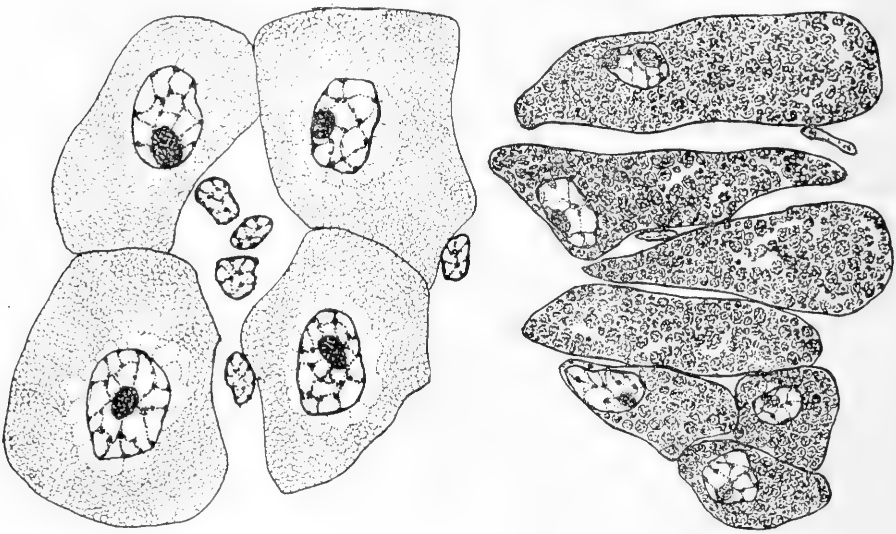

Fig. E Cells from youngest corpus luteum of cow. (× 950.) Compare figure 18.

Fig. F Cells from older corpus luteum of cow, showing pigment developed in cells. (× 950.) Compare figure 19 and figure 21.

The next to last corpus luteum is much reduced in size. Its color has lost the pinkish shade and it appears a solid bright yellow. This is also soluble in absolute alcohol and xylol as in the first stage. The cells and nuclei both look a little shrunken. In one cow, this second corpus luteum contained a few amorphous yellow particles like those described for the hen.

In the third oldest corpus luteum, the tissue is shrunken so that a mere speck shows on the surface. This is the stage resembling the yellow spots of the hen's ovary. Dissection shows that it is reduced in all diameters. That part of this decrease in size is due to cell shrinkage is well demonstrated by comparison of figures E and F, which are drawn to the same scale. Not only the nucleus but the cell body is at least halved in size. The color now is darker, being a brick red. This is not due to blood vessels, as sections do not show any more than formerly. It is due to the development of a dark yellow pigment, the same substance which appeared in small quantity in the younger corpus luteum and in large quantity in the hen ovary. In this stage of involution it is developed in large quantities, practically filling up many of the lutear cells (figs. 19 and 21 and fig. F). In unstained sections it gives a yellow color to most of the section.

In the fourth oldest corpus luteum of the two series and in the scattered older ones sectioned, the structure is similar to that in the third oldest, the yellow amorphous masses being possibly larger and more distinct.

This yellow material certainly looks the same as that in the hen ovary. The chief structural difference is that it is all confined within cells with distinct cell walls in the cow, while in the bird, the cells forming it, lose their boundaries and the particles are formed in a vacuolated network with scattered shrunken nuclei (cf. figs. 20 and 21).

Sudan III reacts similarly with hand sections of formalin material from both cow and hen ovary. All four stages in the cow series take the red color showing the presence of a fatty substance in the cell. This corroborates the evidence from the solvent action of absolute alcohol and xylol. But in the third and fourth stages, yellow amorphous pigment particles can be seen glistening in the red background. The pigment is not of fatty nature in the cow, any more than it is in the hen. In fact, this substance is so similar in the two animals, that we shall from now on speak of a corpus luteum in the hen, and call the cells forming this pigment, lutear cells.

This development of a non-fatty pigment in the mammalian lutear cells has been already described by Mulon as occurring in atretic follicles. He speaks of the lipocholesterine as changing over to an indelible pigment. This same substance certainly forms in the involution of the corpus luteum of a discharged follicle as shown in this present work.

It is of especial interest to find that Blair Bell's description of the corpus luteum in Ornithorhynchus, a primitive oviparous mammal, shows it very much like that in the hen. It often remains hollow, it never becomes very large. It consists chiefly of a thickened theca interna. Sometimes it becomes a solid fibrous mass. One of Bell's figures almost exactly resembles figure 4 of this paper. One would like to know whether the yellow pigment is found in Ornithorhynchus thus making its resemblance to the bird even more striking.

V. BIOCHEMICAL CHARACTER OF PIGMENT OF CORPUS LUTEUM

The identity of this yellow amorphous pigment in the corpus luteum remains in the ovary of the hen and of the cow has been put to chemical tests as well as morphological; first of a microchemical nature, as already partially described, and secondly by various special chemical solvents. The work of Escher and of Palmer and Eckles on animal pigments has been consulted in selecting the reagents to use.

The microchemical tests have been discussed in previous sections, but will be summarized here. Microscopical technique processes have shown the identical behavior of the pigment in hen and cow. It does not dissolve in alcohol or oils. It will not stain with basic nuclear stains such as haematoxylin and Kresylviolet, or with acid counterstains, such as eosin, methyl blue, anilin blue, orange G, or with such a stain as iron haematoxylin. Neither does it stain with the fat stain, Sudan III, although there may be much fatty material in the cell in which it lies. As normal secretion granules of a protein nature take acid stains and secretion granules of a fatty nature take Sudan III, this pigment is neither protein nor fat in composition.

A further test of its chemical nature was made by trying some of the various solvents used by Escher and by Palmer. Sections were cut in paraffine and mounted on slides and then the paraffine removed by xylol and the sections treated with different chemicals. This pigment is not the carotin described by Palmer, but we could not reach any conclusion as to its chemical nature, as nothing could be found to dissolve it. But the fact of the identity of this pigment in the hen and cow is proven beyond a doubt. Concentrated HCl, HNO_3 and H_2SO_4 were tried and had no effect except that the H_2SO_4 turned the particles dark brown and made them even more distinct than before. For an alkali solvent, strong KOH was used; it turned the pigment bright orange but did not dissolve it. In addition to these various other solvents were tried after consultation with the chemistry department, petroleum ether, sulphuric ether, acetone, carbon bisulphide, and carbon tetrachloride, but none of these had the slightest solvent effect on the pigment. Acetone cleared the background and this made the particles stand out more sharply. Carbon bilsulphide was allowed to act for several hours, but the preparations still contained the pigment at the end of that time in undiminished degree. We conclude that any two substances which can withstand the action of as many well known solvents of as many different properties as this list includes must be of very similar chemical nature. This gives us one more proof that the yellow particles in the hen ovary are the same as those in involuted mammalian corpora lutea.

VI. CHANGES IN ATRETIC FOLLICLES IN THE HEN'S OVARY

Among the developing yolks and discharged follicles of the hen ovary are many degenerating eggs. They can be distinguish d from developing eggs by the shrunken appearance as though the contents did not quite fill out the follicle. Eggs may start to degenerate at different stages. The largest one on the Barred Plymouth Rock ovary was 12 mm. in diameter. Many of them show dark spots which are masses of coagulated blood. Mostly they are smaller than this when involution begins. The

degree of shrinkage shows whether the involution process had recently begun or not. When these degenerating eggs are cut open, the contents is found to be in a more or less fluid state. When these atretic follicles have become reduced in size to 2 or 3 mm., it is no longer possible to distinguish them externally from the discharged follicles; the same kind of a yellow pigment appears in the center.

Studied microscopically, the chief difference between atretic and discharged follicles is that the former have a more distinct cavity which becomes obliterated chiefly by migration of lutear cells into it instead of by shrinkage of the walls. The granulosa is shed similarly. There must frequently be hemorrhage as corpuscles are often found in the cavity. The varying quantity of yolk spheres is one indication of the degree of involution, also the number of lutear cells in the cavity. Figure 12 is an atretic follicle with considerable yolk still unabsorbed. A few lutear cells have filled in to the cavity (fig. 13, *1*). It is particularly clear here that the cells inside of the inner margin of the theca interna are the same in structure as those of epithelial nature in the interna theca. This is just as Benthin describes it for the atretic mammalian follicles. Figures 14 and 15 show a later stage where the yolk is almost all absorbed and the cavity is filled with lutear cells.

Not until the cavity is filled with lutear cells does the yellow pigment already described in discharged follicles, make its appearance. It forms in the lutear cells of atretic follicles in a similar way to that in the discharged follicle. The cell boundaries are possibly not obliterated so completely, so that the morphological resemblance to the cow corpus luteum remains is even more striking than in the case of the discharged follicles. Figure 16 is part of an atretic follicle where the cells are filled with pigment. The amorphous character of this material shows in figure 17 a part of figure 16 under higher magnification.

It is of interest to notice that the lutear cells in the hen in both discharged and atretic follicles originate entirely from the theca interna. In mammals the origin of the lutear cells is a mooted question. Some authors, as Niskoubina, hold that they

have a double origin, from granulosa and theca interna, while others such as Benthin and Hegar, claim that they all come from the theca interna. This point is perfectly clear in birds due to the ease with which one can distinguish these peculiar cells in the internal theca of undischarged follicles and follow them to the thickened mass in the center of the discharged follicles, and see them migrating out into the cavity of the atretic follicles.

The formation of a corpus luteum in atretic as well as discharged follicles makes it possible to identify ovarian tissue in ovaries too abnormal to have ovulated any eggs. Most of the literature of the mammalian ovary considers the involution of the atretic follicle as something distinct from that of the discharged follicle. The mass forming in the atretic follicle is called the corpus atreticum or fibrosum in contradistinction to the corpus luteum. However, Hegar says that it is hard to tell one from the other. They are practically identical in the hen.

VII. SUMMARY

We are now in a position to sum up the points proving the homology of the corpus luteum in the hen and in the cow. There has been much discussion about the origin of the corpus luteum in mammals. In the hen there is no question but that the origin is simply from the theca interna.

The course of development in the hen corpus luteum is an abbreviation or fore-shortening of that in the cow. It corresponds directly to the late involution stages of the cow corpus luteum. They both contain a yellow fatty substance, as shown by the Sudan III, absolute alcohol and xylol reactions. There develops in both a yellow amorphous pigment in the cells containing the fatty substance. This pigment is similar chemically in that it will not stain with basic or acid stains; also in that it will not dissolve in any of the usual solvents, acid alkali or oil.

In the hen, a corpus luteum forms in both discharged and atretic follicles.

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PLATES

DESCRIPTION OF PLATES

We wish to take this occasion to acknowledge our indebtedness to Mr. Royden Hammond for all the photomicrographs, and to Mrs. Maud DeWitt Pearl for the paintings on plate 9.

PLATE 1

EXPLANATION OF FIGURES

1 Medium sized oocyte in hen ovary (× 40), showing three layers to the follicle, the granulosa (*g*), theca interna (*i*), and theca externa (*e*), with nests of lutear cells in the theca interna (*l*).

2 Part of follicle wall in figure 1 at greater magnification (× 352). Labels the same as in figure 1.


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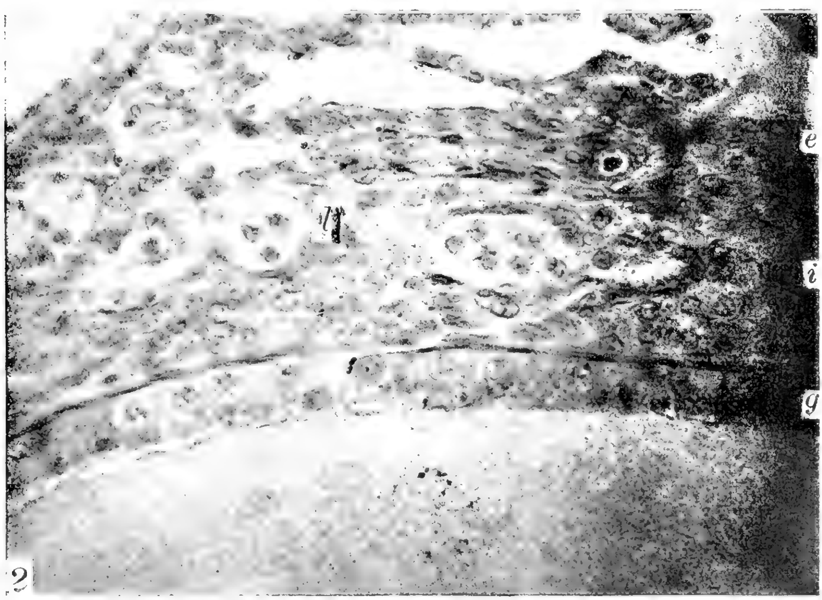
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PLATE 2

EXPLANATION OF FIGURES

3 Young oocytes in hen ovary (× 352), with follicles consisting of a single layer of granulosa (*g*). Nests of lutear cells in the stroma nearby (*l*).

4 Portion of last discharged follicle in hen with thickened theca interna (*i*) and granulosa (*g*) being sloughed off into the cavity. (× 40.)

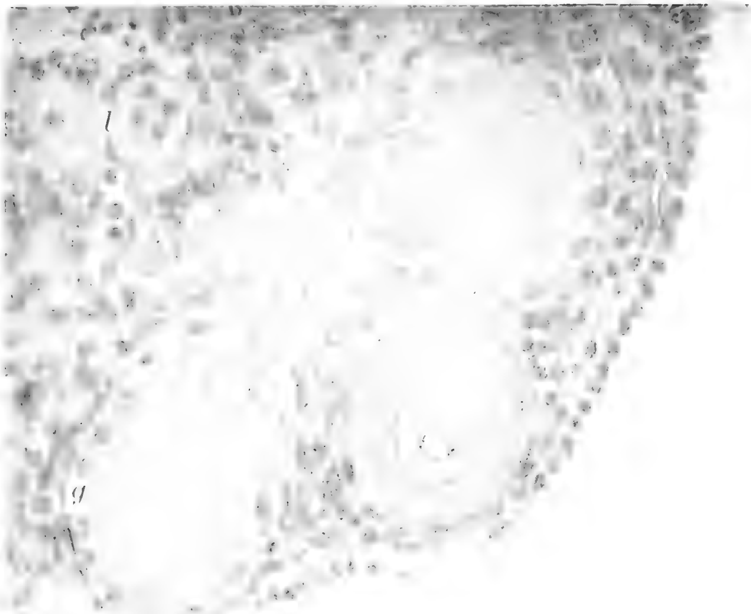

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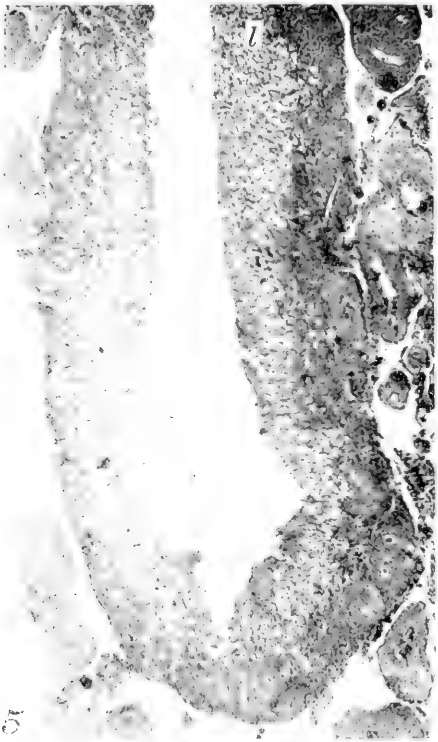
PLATE 3

EXPLANATION OF FIGURES

5 Portion of sixth from last discharged follicle, showing large number of lutear cells (*l*) in the theca interna ($\times$ 40).

6 Part of figure 5 enlarged ($\times$ 176).

7 Small discharged follicle with cavity nearly obliterated. Small plug of cells (*p*), filling in the cavity. Theca interna filled with masses of lutear cells (*l*). ($\times$ 40.)



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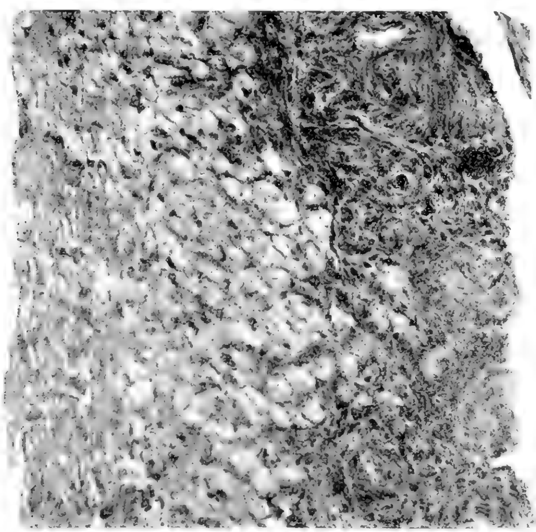

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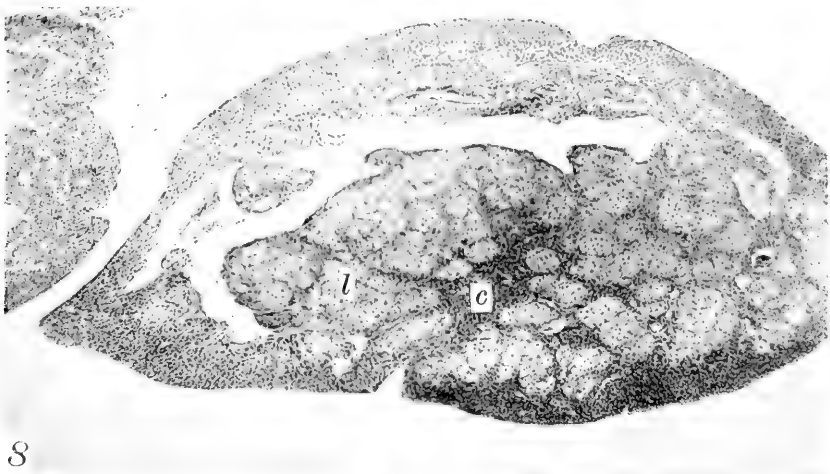
PLATE 4

EXPLANATION OF FIGURES

8 Discharged follicle with cavity completely obliterated. The chief component is masses of lutear cells (*l*). The connective tissue center represents original location of cavity (*c*). × 40.

9 Part of figure 8 at greater magnification (× 176), showing interstitial cells (*i.c.*) in connective tissue between lutear masses.



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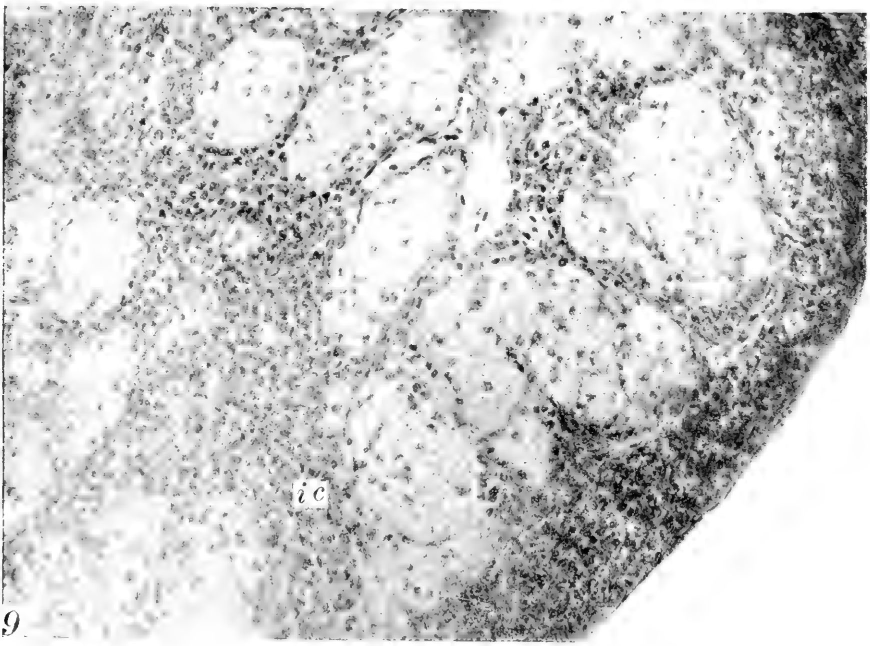

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PLATE 5

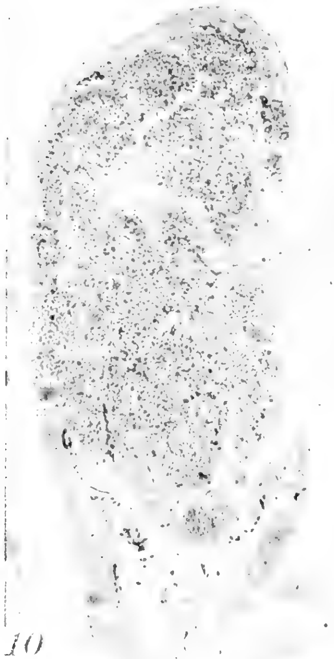
EXPLANATION OF FIGURES

10 Later stage of solid discharged follicle, showing large development of yellow pigment in lutear masses (× 40).

11 Part of figure 10 at greater magnification (× 176), showing pigment particles.

12 Atretic follicles in hen ovary, with yolk spheres in central cavity (× 40).

13 Part of figure 12 at greater magnification (× 176). Lutear cells (*l*) show in theca interna and also among yolk spheres in the cavity.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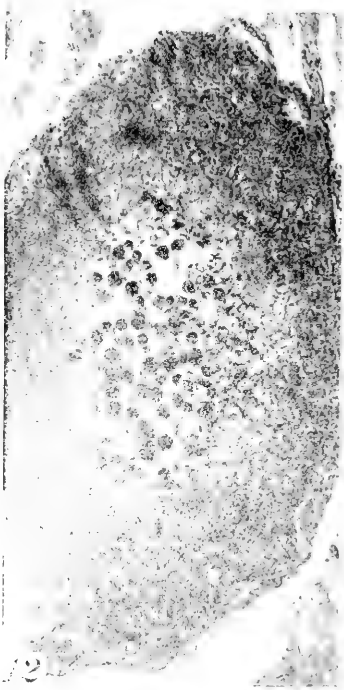
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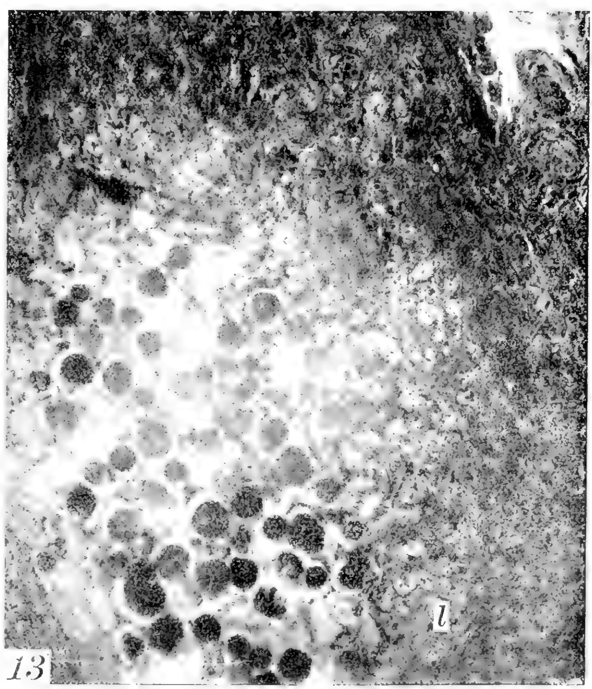
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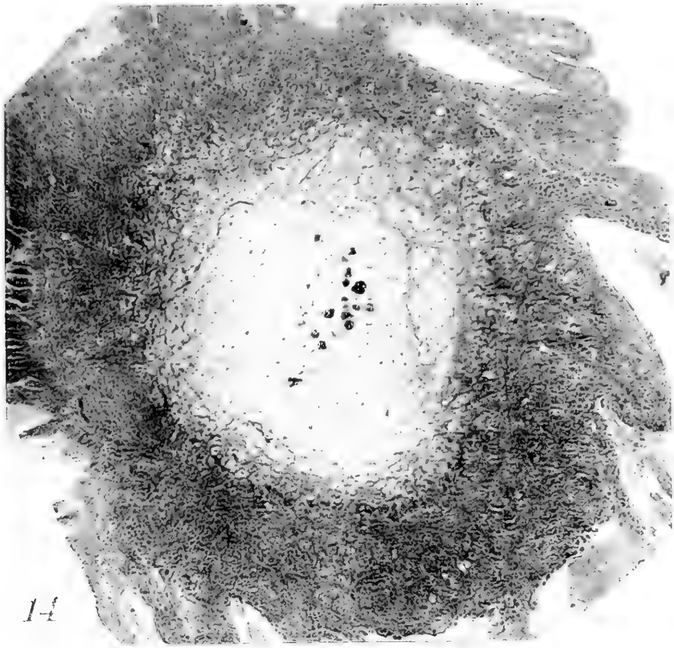
PLATE 6

EXPLANATION OF FIGURES

14 Later stage of atretic follicle (× 40). Only a few yolk spheres remain in cavity. Cavity is practically filled with lutear cells.

15 Part of figure 14 at greater magnification (× 176), showing lutear cells in theca interna (*i*), as well as the central cavity.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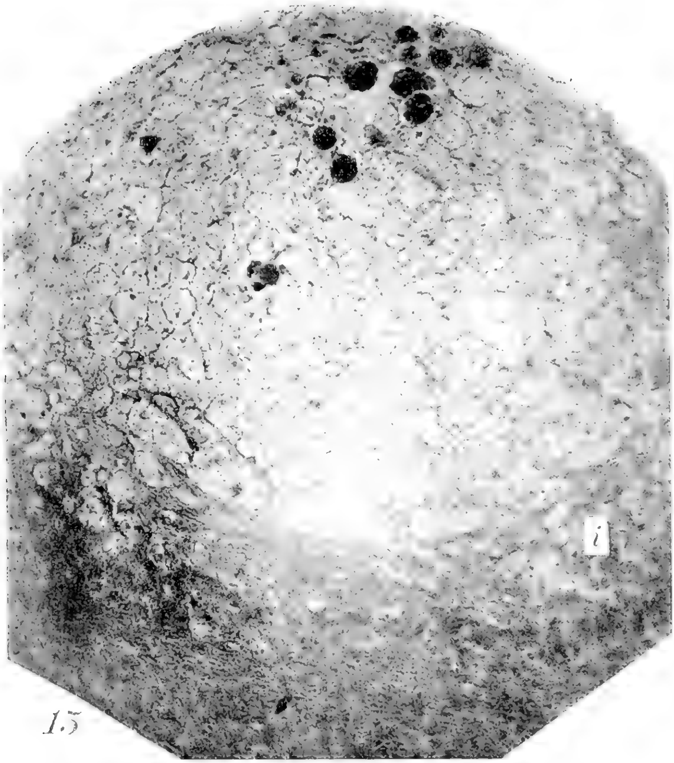
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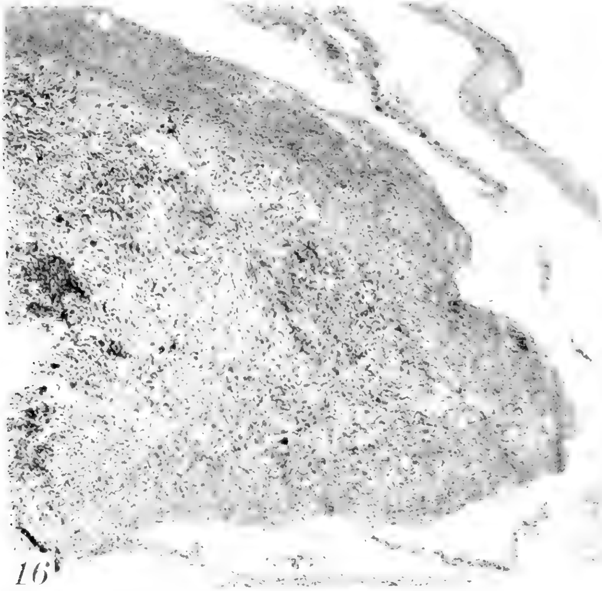
PLATE 7

EXPLANATION OF FIGURES

16 Atretic follicle in which the pigment particles have developed in the lutear cells (× 40).

17 Part of figure 16 (× 176).



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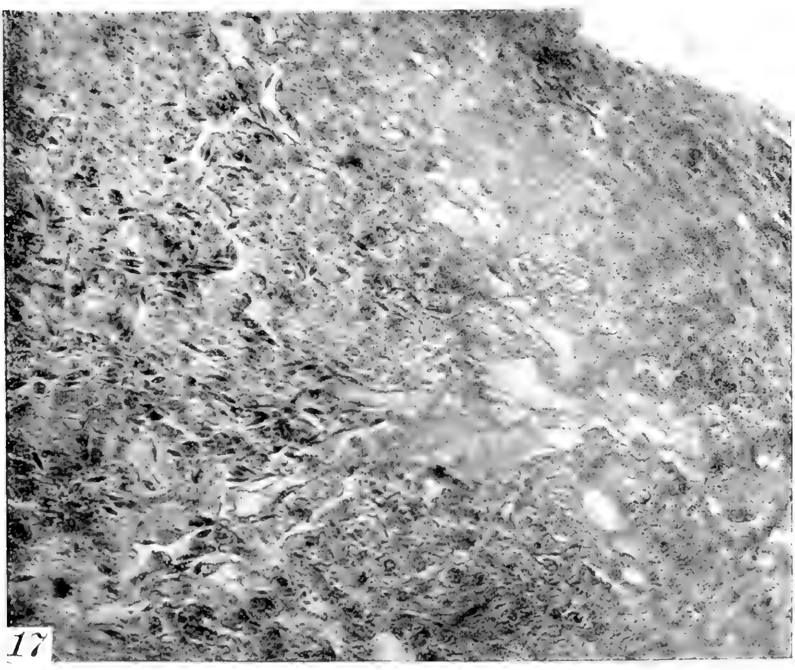

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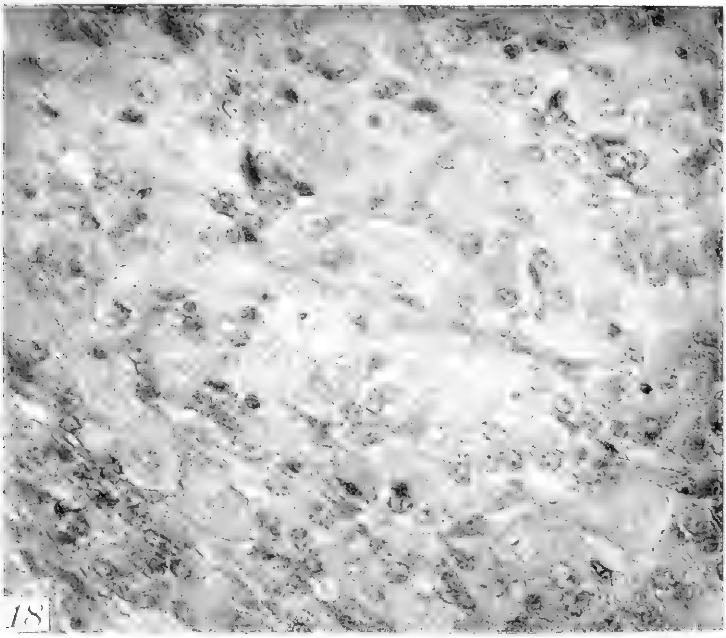
PLATE 8

EXPLANATION OF FIGURES

18 Section of youngest corpus luteum of cow (× 176).

19 Section of older corpus luteum of cow (× 176), showing cells filled with pigment particles.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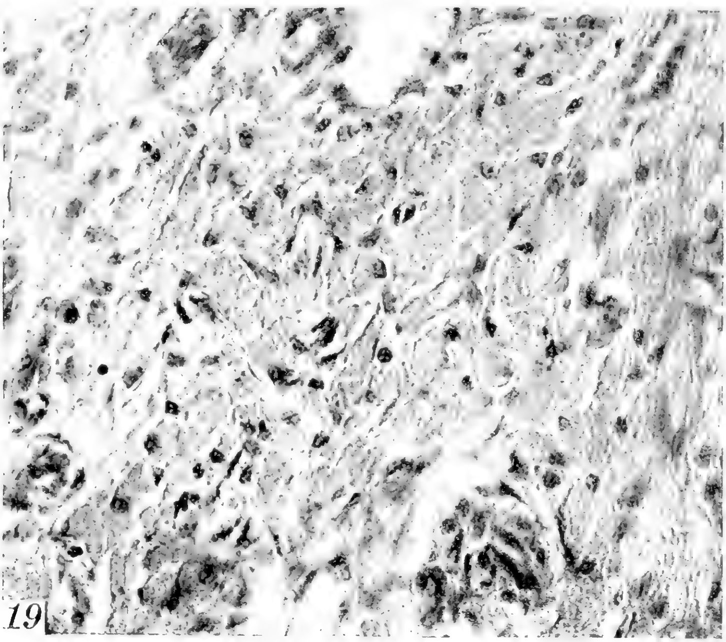
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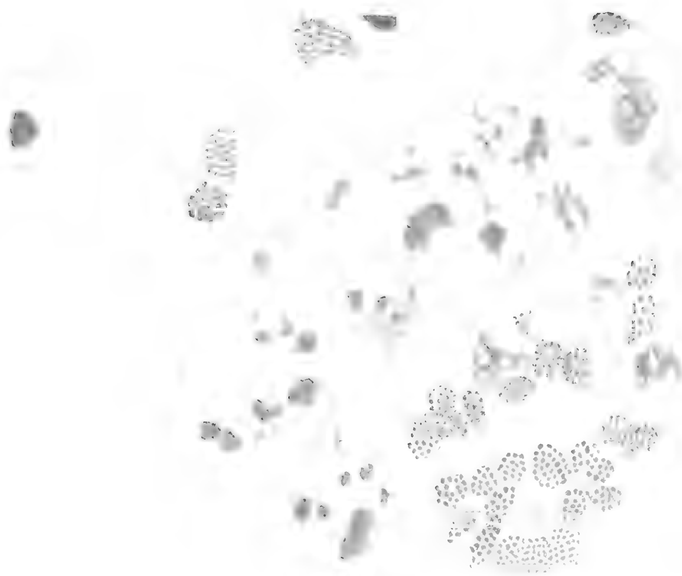
PLATE 9

EXPLANATION OF FIGURES

20 Section of discharged follicle of hen ovary, stained in Mallory's stain. Connective tissue = blue. Corpuscle = red. Interstitial cells = purple. Lutear pigment = yellow.

21 Section of older corpus luteum of cow, stained in Mallory's stain. Tissues colored as in figure 20.



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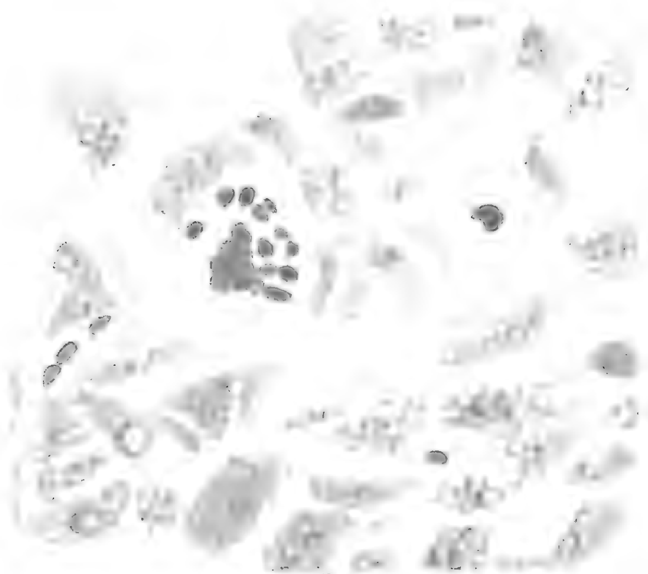

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
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STUDIES ON THE GROWTH OF BLOOD-VESSELS IN THE TAIL OF THE FROG LARVA—BY OBSERVATION AND EXPERIMENT ON THE LIVING ANIMAL

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SIXTEEN FIGURES

These studies were begun and part of them were made in the laboratory and under the inspiration of my beloved teacher and master, the late Professor Franklin P. Mall, and it is with a sense of the deepest gratitude and reverence that I acknowledge the immeasurable debt which I owe to him.

INTRODUCTION

The development of the vascular system falls broadly into two stages: (1) the stage of primary differentiation, or histogenesis, and (2) the stage of extension and elaboration of arteries, veins, and capillaries. The exact manner and place, in which the primary differentiation occurs is an unsettled problem, and is, at the present time, the subject of spirited controversy. It has not been satisfactorily decided whether blood-vessel endothelium differentiates from entoderm, or mesoderm—and if from mesoderm, whether from mesenchyme generally or from the mesothelial lining of the coelom. Nor has it been determined whether this primary differentiation occurs on the walls of the yolk sac alone, or in the embryo proper, or whether it may take place both on the yolk sac and in the embryo. Another unsettled point is the extent of time over which the primary differentiation takes place. Recent discussions and observations supporting one or another of these views may be found in the following: Minot ('12), Evans ('12), Rückert and Mollier ('06), Schulte ('14), Bremer ('14), Stockard ('15 A), Reagan ('17), Sabin ('17).

The second stage of vascular development includes the further

extension and development of the system after the primary differentiation has taken place and after the circulation has been established, and it is with this second stage that the studies here reported are concerned. In this stage, which continues throughout life, the vascular endothelium spreads through the growing organism, arteries and veins develop, until the extensive and complicated vascular system of the adult is perfected. It is principally characterized by the formation of new vessels by the sending out of sprouts from the vessels already present, instead of by the transformation of mesenchyme, of other undifferentiated cells, as in the first stage, and by the action on the vessels of the mechanical and chemical factors concerned with the circulation of blood and interchange of substances through the wall.

In spite of the abundant evidence in favor of this mode of spreading of the vascular endothelium, after its primary differentiation, there are observers who adhere to the view that at any time throughout life, mesenchyme (or other undifferentiated cells) may be transformed into vascular endothelium. This view is held by Maxinow, Weidenreich, and Mollier (cf. discussion in Schulte, '14,) who believe that, not only may reticulum cells and leucocytes be transformed into blood-vessel endothelium, but that the reverse transformation may take place—in brief, that vascular endothelium is not a specific tissue, but is interchangeable with the other tissues mentioned. The evidence for this view has in no case been conclusive. It is clear, however, that the manifestation of the property of sprouting does not form a sharp boundary line in time of development between stages, for apparently sprouting commences before the differentiation of endothelium is everywhere complete (cf. Stockard '15, B and Sabin '17). It is probable that the period of overlapping is very short.

It is also clear, particularly from the studies of Miss Sabin ('17), that the development of arteries and veins takes place to some extent before the circulation is established. In chick embryos she found that, before circulation starts, part of the aortae, the two vitelline veins next the heart, parts of the cardinal veins and the duct of Cuvier are clearly present as definite vessels.

That there is a secondary stage in the development of the blood-vascular endothelium, in which the endothelium spreads by sprouting, instead of by the transformation of indifferent cells, has been proven by direct observation. In the transparent fin expansion of the tail of the tad-pole, this process has been watched during life by several observers, especially Golubew ('69), J. Arnold ('71) and Rouget ('73), who have seen blood capillaries send out sprouts, which extended until they met and anastomosed with other sprouts or capillaries and into which a lumen advanced—all without the interposition of outside cells. This view is supported also, among others, by J. Meyer ('53), Bobritzky ('85), His ('69), Kölliker ('86), R. Thoma ('93), Marchand ('01), Ziegler ('05) and Evans ('09 A). While this mode of growth has not been proven by direct observation for all vessels in all animals, and while the existence of other modes of extension is perhaps not necessarily excluded, it is a fair hypothesis that this is the universal mode of spreading of the vascular endothelium, once it has differentiated, and cannot be abandoned until more convincing objections are brought than have been produced up to the present time.

It is not the primary purpose of the present study to enter either into the problem as to the time, in embryonic development, at which the second stage begins, nor the problem whether growth by sprouting is the universal mode of spreading during this period. It is rather to consider the problem as to how, in a region where, and at a time in development when growth by sprouting has been repeatedly verified, and after the circulation has become established, the capillaries are transformed into arteries and veins; to study the modes of action and reaction of endothelium—the laws which regulate its growth.

Such a study is by no means new, for it has been, through many years of fruitful investigations, the object of W. Roux and particularly of R. Thoma and numerous coworkers to discover the factors which regulate the growth of vessels, while many others, including Nothnagel ('84), Mall ('06), Evans ('09, A and B, '12) have studied the same problem less extensively.

The initial stimulus to this study was given by W. Roux ('79), in his Inaugural Dissertation, in which he studied the "angle of branching" in relation to the relative size of the branch, and the shape of vessels in the neighborhood of a branch. He found that this angle, which lies between a line continuing the axis of the main stem and the axis of the branch, varies with the relative size of the branch—that, in general, the larger (relatively) the branch, the smaller the angle, and the smaller the branch, the larger the angle. He also found that the lumen of an artery shows a widening with subsequent narrowing immediately after branching, and that the opening of the branch is oval rather than round. By experiments with openings made in vessels and in tubes and with the use of malleable substances such as lard placed in such openings and on the interior of tubes, he found that the direction taken and the shape found is, in the case of the artery, practically the same as the shape and direction of the stream of fluid emerging from openings in vessels and tubes. He concluded that the shape and direction of arteries at the place of branching are determined by the action of hemodynamical factors; that the blood-vessel wall responds by taking the shape which allows a minimum of friction. The general and important conclusion was that the size and shape of arteries and veins, in the growing and adult animal, are regulated, not by heredity, but by the action of mechanical factors.

Thoma's conclusions were based mainly on studies made on the extra-embryonic yolk sac vessels of chick embryos. From a series of injections he found that there is formed, first, an indifferent plexus of capillaries, interposed between the aorta and the venous end of the heart, and that out of this plexus, those vessels which are so placed as to have the greatest amount of blood flowing through them enlarge to become arteries and veins, while others remain capillaries, or atrophy.

The results of these and other studies by Thoma ('11) may be briefly summarized. He finds that blood-vessels are regulated in their growth by mechanical factors, which he expresses in the form of 'laws' ('Histomechaniche Principien'), as follows:

1. Das Wachstum des queren Durchmessers, also des Gefässlichtung ist abhängig von der Geschwindigkeit des Blutstromes. Dasselbe beginnt, sowie die Stromgeschwindigkeit der nahe an der Gefässwand strömenden Blutschichten einen Schwellenwert überschreitet, den ich mit U bezeichnen will, und ist innerhalb gewisser Grenzen ein um so rascheres, je so mehr die Stromgeschwindigkeit über den Schwellenwert U, hinaus zunimmt. Dagegen tritt ein negatives Wachstum, eine Abname des Gefässumfanges ein, wenn die Geschwindigkeit der nahe an der Gefässwand strömenden Blutschichten kleiner wird als der Schwellenwert v.

2. Das Längenwachstum der Gefässwand ist abhängig von den Zugwirkungen der das Gefäss umgebenden Gewebe und zwar sowohl von denjenigen Zugwirkungen welche das Längenwachstum der umgebenden Gewebe erzeugt als von denjenigen Zugwirkungen, welche bei "Anderungen der Gelenkstellungen eintreten," etc.

3. "Wird das Wachstum der Wanddicke bestimmt durch die Spannung der Gefässwand." This is determined by the blood pressure and the size of the vessel.

4. (proposed as an hypothesis, not yet proven). Die Umbildung von Kapillaren ist abhängig von dem in den Kapillaren herrschenden Blutdruck und stellt such an denjenigen Stellen der Kapillarbezirke ein, an welchen der zwischen dem Kapillarinhalte und der Gewebsflüssigkeit bestehende Druckunterschied einen gewissen Schwellenwert p überschreitet. Dieser Schwellenwert ist jedoch in den verschiedenen Kapillarbezirken je nach den Eigenschaften der die Kapillaren umgebenden Gewebe verschieden gross.

Expressed more simply they are:

1. Increase or decrease in the size of a vessel is regulated by the rate of the blood flow.

2. Increase or decrease in the length of a vessel is governed by the tension exerted on the vessel wall in a longitudinal direction by tissues and organs outside of the vessel.

3. Increase or decrease in the thickness of the vessel wall is dependent upon the blood pressure.

4. New formation of capillaries depends upon increase of pressure in the capillary area (proposed as an hypothesis—not yet proven).

The ultimate controlling factor Thoma considers to lie in the metabolism of the organs ('93, pp. 49–51). It is this which regulates, primarily, the increase or decrease in capillaries, which, in turn, sets in motion the mechanism which results in the increase or decrease in the size of arteries and veins, the increase in strength of heart beat, etc.

Roux, in his later writings, discusses, mainly in a theoretical way, the factors involved in the increase in size of vessels, and the new formation of capillaries. His views as to the new formation of capillaries, expressed briefly in 1895, repeated more fully in 1910, and again repeated, in a controversial article in 1911, are perhaps most completely expressed in 1910, p. 88, where he says:

> Ist der Verbrauch in dem Parenchym, welches eine Kapillare umgibt, einige Zeit dauernd derartig gesteigert, dass aus den vorstehend erörterten Gründen mehr Stoff als normal hindurchtritt, so wird wohl die an der Stelle stärksten Durchtritts gelegene Wandungszelle durch die verstärkte Leistung in der Richtung des Austritts zur Sprössung angeregt. Dasselbe geschieht natürlich auch an der denselben grösseren Parenchymtheil von der andern Seite der umschliessenden und ernährenden Kapillare. Diese noch nicht als Kapillarenfungierenden sprossen treffen, wohl durch chemotropisch vermittelten Cytotropism, aufeinander, also in ähnlicher Weise wie ich es an von mir isolirten Furchungszellen sah, einerlei ob diese Zellen noch freilagen oder schon wieder an etwas anderem (an der Zellen oder am Boden des Gefässes) hafteten. Der vererbte gestaltende Reaktionsmechanismus der Kapillarwand, der zum Hohlwerden und zur weiteren Ausbildung der neuen Kapillaren mit Bildung von Nerven und kontraktilen Elementen führt, wird auf diese Weise aktiviert und so eine neue funktionsfäbing Kapillare gebildet.

Like Thoma, Roux considers the metabolism of the tissue the primary factor in new growth of capillaries. As for the specific stimulus, however, he disagrees. According to Thoma, increased metabolism causes increase in blood pressure in the capillary area, to which the endothelium is thought to respond by sending out sprouts, while Roux' view is that the new sprout is sent out as a direct response on the part of the endothelial cell to the passage through it of an increased amount of substances. In criticism of Thoma's hypothesis, Roux ('11, p. 201) calls attention to the absence of any noticeable new formation of capillaries in tricuspid or mitral insufficiency, in which conditions there is a rise in blood-pressure in the capillaries.

Thoma's first histomechanical law that the size of the vessel is regulated by the rate of blood flow, is criticized by Roux chiefly because he can see no way in which the moving stream can affect the wall, since, as first shown by Helmholtz, there is

a thin layer of fluid next the wall which is immovable. His explanation for growth in size of vessels is that it is brought about through the agency of the vasomotor nervous mechanism; that, following increased metabolism and formation of new capillaries, there is a reflex widening of the arteries and possibly also of the veins of the affected region. This widening, if continued long enough, results in a permanent adaptation of the vessel wall to the increased volume of blood by growth processes.

Roux apparently agrees with Thoma's law as to the increase in thickness of the vessel wall.

Mall ('06), in an extensive review and discussion of Thoma's histomechanical laws, finds support for Thoma's first law, in his studies on the growth of glands. Like Roux, however, he disagrees with Thoma in his hypothesis that the formation of capillaries is dependent on increase in blood-pressure in the capillary area. "In reality," he says (p. 250), "we can only state definitely that with the new formation of tissue new blood-vessels may grow into it, for all new tissue does not have blood-vessels." The precise stimulus for the formation of capillaries is unknown. Again (p. 251), he says, "The first and guiding blood-vessel is the capillary, which grows in all directions, forming a plexus. Secondary changes made arteries and veins of them and their laws of growth have been discovered and clearly stated by Thoma."

It has been shown by a series of investigators—among them—Erick Müller ('03, '04), Rabl ('07), Bremer ('12) and H. Smith ('09), and particularly Evans ('09, A and B) that many of the larger arteries and veins in the body of the developing embryo are first formed as capillaries, which grow as irregular plexuses, and out of which certain ones are differentiated to form arteries and veins. Evans, who has made the most extensive studies in this field, has described the caudal portion of the aorta, the chief veins, the pulmonary, subclavian and sciatic arteries as developing in this manner. He concludes that the histomechanical laws of Thoma are the factors which govern the process.

A number of investigators have suggested that new capillaries are formed as the result of the action of specific 'chemiotactic'

(better 'hemangiotactic') substances outside the capillaries. According to Marchand ('01, p. 148), Leber ('88) first suggested this explanation, to which Marchand is slightly inclined. It was suggested again by J. Loeb ('93) as an explanation for the growth of vessels in fish embryos whose heart action was eliminated by the action of chemical substances. Evans makes a similar suggestion. In each case it has been proposed merely as a tentative hypothesis and has not been tested.

Over against this group of investigators whose studies have gone to show that blood-vessels are regulated in their growth by the action of mechanical and chemical factors, and some of whom have attempted to define this regulation in terms of specific laws of growth, there are others who have supported the view that mechanical factors play little if any part in determining the formation of arteries and veins, and who attribute it rather to the action of hereditary influences. Possibly the strongest adherent of this view is Hochstetter, who has made so many important studies on the comparative anatomy of the vascular system. His view is probably most concisely presented by his pupil, Elze ('12) in an article criticizing the conclusions of Evans and Thoma. In brief, it is that the primitive form of the vascular system is not a capillary plexus, but a single artery and vein, such as is formed in the limbs and digits of amphibians, and also in the segmental arteries; while capillary plexuses are secondary formations.

Now it is interesting that support for this view has come in part from the two men who have been most prominent in advocating the regulating action of mechanical factors, namely, Thoma and Roux. Thoma ('93, p. 28) mentions that the aorta is developed as a definite vessel before the heart commences to beat, while Roux emphasizes a first stage in the development of the vascular system, as of other systems, in which differentiation and growth take place as a result of heredity (preformation)—a stage which includes the formation of 'the anlage of the typically laid down chief vascular stems' ('95, pp. 326–7, footnote). Roux bases this conclusion on chance observations made on the area vasculosa of chick embryos, in which the embryo failed to

develop, but in which vessels, including the border vein and some others differentiated in situations corresponding with the normal. That growth of capillaries and larger vessels in embryos is regulated not entirely by the metabolism of the tissues, but in part at least by hereditary influences, is shown, he believes, by the richness of the capillary plexus in the lung and the relatively great size of the pulmonary arteries and veins, which, 'according to Wiener,' are, before birth, four to six times as large as the weight of the lung tissue justifies, in comparison with other organs. Wiener studied the proportion between size of artery and weight of organ.

Support is lent to this view by the results of studies made on embryos whose heart-beat has been eliminated experimentally either by mechanical removal or by chemical inhibition. Dareste ('77) J. Loeb ('93), Patterson ('09), Knower ('07) and Stockard ('15 A) agree in finding certain typical arteries and veins formed in such embryos, in which the mechanical action of the circulation has been eliminated—in fish, frog and chick embryos.

The indications are that the truth lies between the two extreme views; that what we are forced to call hereditary factors do play a part, not only in the primary differentiation of blood-vascular endothelium and its capacity for growth by sprouting, but in the formation of some of the main vessels in the embryo (how great a part and how long exerted in embryonic life, has not yet been cleared up, cf, Miss Sabin, '17, previously referred to) that, on the other hand, the vascular system does become, at an early stage, dependent, at least to a very great extent, upon the regulative action of mechanical and chemical forces.

Were it found that arteries and veins in latter stages are completely regulated as regards diameter, length, thickness of wall, and position by the action of mechanical and chemical factors, it would be quite compatible with our knowledge of the development of other tissues and organs, to find that a crude pattern of such mechanically controlled structures should reappear in the embryo (Thoma, '93, p. 28).

As to the precise nature of the mechanical and chemical factors which regulate the growth of the vascular endothelium,

there is, as the foregoing review and discussion shows, difference of opinion sufficient to justify further observation and experiment.

METHODS USED IN PRESENT STUDIES

Since most of the studies referred to were made on successive stages, usually of injected embryos, in fixed preparations, it seemed that it would be worth while to study the changes in the same vessels of the same living embryo, following certain vessels through the critical stages in their development, keeping records of the circulatory conditions, and of all changes in the size of the vessels, and the direction of the angle of branching, et cetera. For such a study the transparent fin expansion of the tail of frog larvae is admirably adapted, for a larva can, by the use of chloretone as an anesthetic, be kept under observation over a period of weeks, and careful camera lucida records made as frequently as desired. Since the chloretone interferes but little with the heart beat, records can also be kept of the circulatory conditions in each of the vessels which is being watched. (For details of the method used see E. R. Clark ('12).) In the most extensive series of studies made on a single tad-pole, the observations were started when the larva (rana sylvatica) first became transparent enough to enable the course of the vessels in the dorsal fin to be made out, and records were made at daily intervals, at first, when new formation of vessels was most rapid, later, when changes were slower, at considerably longer intervals. During the observations the larva increased in length from 10.5 mm. to 29 mm. There was thus procured a record giving the vascular changes, with notes as to the condition of circulation in each vessel for a considerable section of the fin, from a stage at which the entire system consisted of a few capillary loops, to a stage in which a fairly complicated system of arterioles, capillaries and venules had developed. In addition to this series of studies, numerous shorter studies were made, on larvae of r. sylvatica, r. palustris and r. catesbiana. Brief reference has been made in an earlier paper (E. R. Clark ('09)) to the blood-vessel changes in the tail of the frog larva, and some of the matter

included in the present study was presented at the meeting of the Am. Ass. of Anat. in 1914 (E. R. Clark ('15),) where drawings were shown.

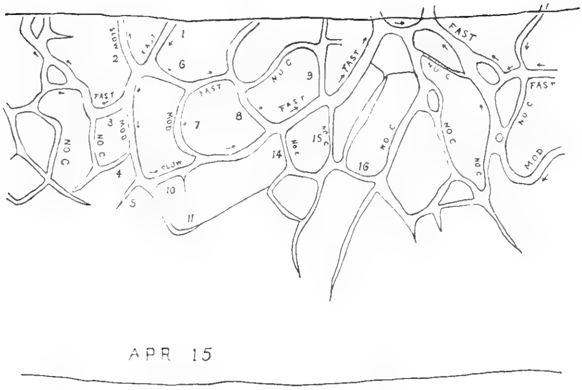
DESCRIPTION OF FINDINGS

When the blood-vessels in the dorsal fin of r. sylvatica larvae first become clearly visible, owing to the absorption of some of the yolk and pigment present in young larvae, they form a system of capillary loops, making an irregular meshwork of rather wide vessels, all connected with one another. On the arterial side they are connected with the main caudal artery, and on the venous side with the main caudal vein, which are located ventral to the notocord, and between the two layers of myotomes. The vessels reach the dorsal fin by passing dorsally between the notocord and spinal cord in the center and the layers of myotomes on either side. With the low power of the microscope their course may be easily followed from the main caudal vessels to their emergence from between the myotomes. With the higher power this is more difficult, and in most of the studies made, only the vessels in the dorsal fin proper, after their emergence from between the myotomes, are drawn.

While in many of the studies all the vessels iu the dorsal fin have been followed, a small area is selected for closer study, and for reproduction, because any section illustrates the fundamental principles involved in blood-vessel development. In the series which is reproduced an area was chosen which included an arteriole and a venule and the region between the two, as well as a part of the regions on either side. This area is sufficiently large to make it possible to follow the changes introduced by the development of new capillaries on the vessels already present.

The changes which occur in such a selected area are shown in figures 1 to 8, and will now be taken up in detail and analyzed.

There is present in the first record a very simple type of circulation. An arteriole, or, perhaps better, an arterial capillary is seen toward the left. Two branches are given off from this vessel on the right, and two on the left, through which blood corpuscles are circulating. In addition there is a third branch



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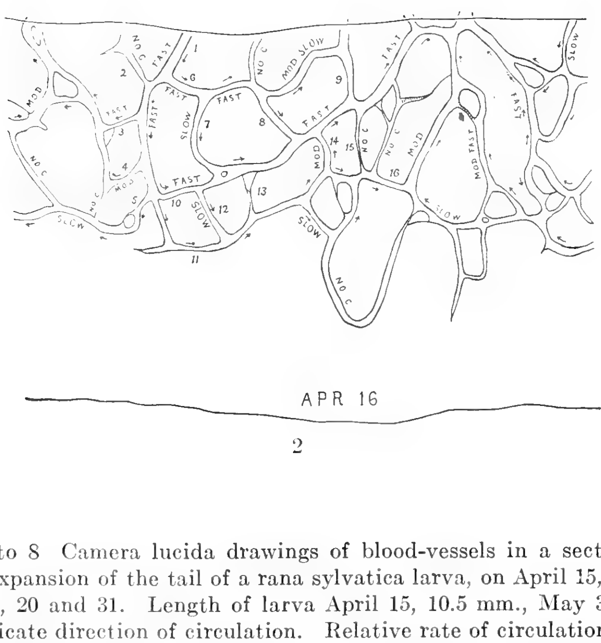

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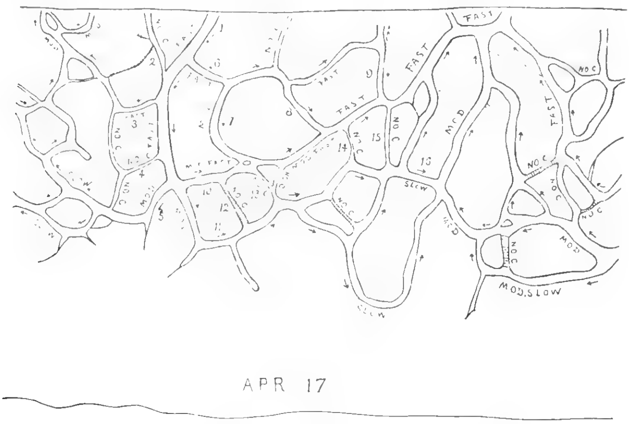
Figs. 1 to 8 Camera lucida drawings of blood-vessels in a section of the dorsal fin expansion of the tail of a rana sylvatica larva, on April 15, 16, 17, 18, 20, May 12, 20 and 31. Length of larva April 15, 10.5 mm., May 31, 29 mm. Arrows indicate direction of circulation. Relative rate of circulation indicated as *FAST*, *MOD.*, moderate, *SLOW*, *NO C.*, no circulation. Corresponding vessels are numbered. Vessels present in one drawing which have been retracted in the next are cross-hatched. The positions formerly occupied by vessels which have retracted are indicated by dotted lines. In figure 8, the vessels which were present in figure 1 are stippled. enl. (1:50).

on the left, with a continuous lumen, but without circulation, and a fourth which ends blindly. The arteriole ends with a bend to the right, from which a long thread extends to another non-circulating vessel. Following the two branches to the right, it is seen that the first pursues a winding course, while the second passes fairly directly to the main venule or venous capillary, near the right. Between the two branches are three communicating vessels, the last of which forms a non-circulating loop. Below the venule there is a rather elaborate plexus of vessels



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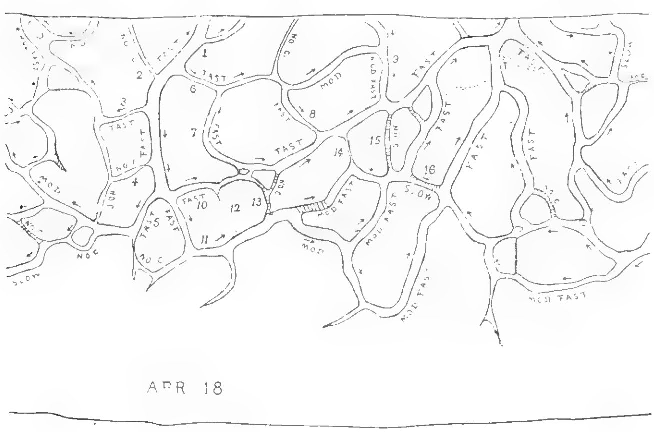

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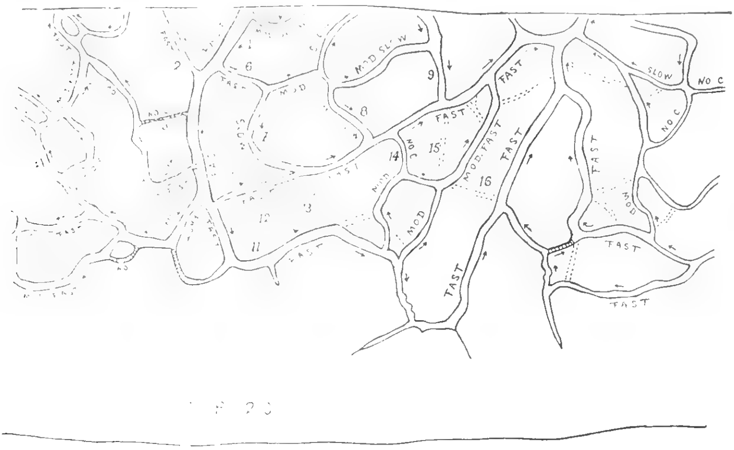
containing, for the most part, lumina, but without circulation. Extending peripherally from them are several blind-ending projections. As regards the rate of circulation, through branch 6 it is relatively 'fast,' while through branch 10 it is 'slow.' To summarize the condition of the vessels for the small area selected, there is a simple irregular plexus of capillaries with wide and rather irregular lumen, some of them with and some without circulation. For convenience, the principal afferent and efferent capillaries have been called arteriole, or arterial capillary, and venule, or venous capillary, though they are of capillary size and appearance.

In figure 2 (a day later) a 'slow' circulation has started in a number of vessels which had been non-circulating on the previous day, and several new sprouts and connections between sprouts have formed. In figure 3 this has continued, and has been accompanied by an increase in the rate of circulation in some of the vessels. On the other hand, there are some vessels in which the circulation has diminished, or ceased altogether. In figures 4 and 5 the same processes have continued—a slight formation of new vessels, with modification of the rates of circulation in many of the vessels, an increase in some and a decrease in others. in addition a new change has become marked, namely, the disappearance of certain capillaries, in which the circulation had ceased, or in which the circulation had never started.

Owing to the fact that the prolonged use of chloretone had caused a slowing of growth processes, the larva was allowed to develop in fresh water, with observations at less frequent intervals, in order to see the fate of the vessels being watched, after a considerable amount of growth had taken place. A record was made on April 22, which showed very little change from the record of April 20. The succeeding records were made May 12, May 20 and May 31. While these later records are not close enough together to show all the growth changes, they show very well the new capillary areas which have developed, their relation to the vessels already present, and the changes which the earlier formed vessels have undergone, in consequence. Thus it will be

seen that in figure 6 and 7 the non-vascular zone toward the margin of the fin has become much reduced in extent by the formation of new vessels, until only a very narrow non-vascular zone is left. It will also be noted that there has been a general expansion of the tail, so that the meshes between the vessels are not-



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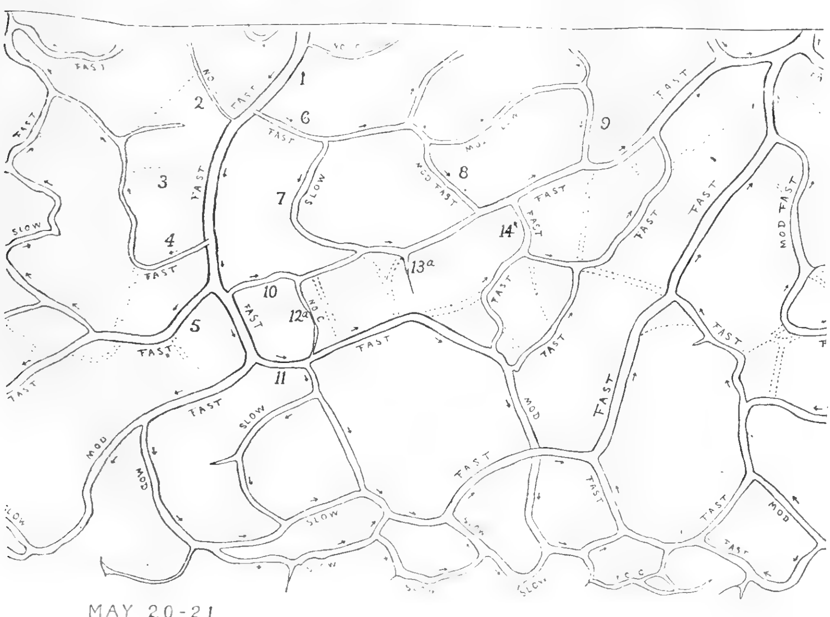
iceably larger, and the vessels longer. The last record shows this enlargement of the tail most markedly. The tail has increased not only in length and height, but also in thickness, and with the enlargement there has been a very great development of new capillaries, in the widened spaces of the blood-vessel meshwork. In the half of the fin next the muscle, where the growth in thickness has been most pronounced, many of the new capillaries are in new planes, more superficial than the earlier formed vessels. As regards the fate of the vessels present in the earlier stages, it is seen that there has been a marked differentiation. In figure 1 the vessels present are nearly all of a uniform diameter. In each successive record there is a progressive differentiation, in which certain capillaries increase in size, others remain of the same, or slightly diminished caliber, while others disappear. In the last stage this differentiation is seen at its maximum; definite arterioles and venules have formed, which supply and drain considerable capillary areas. In this elaborated system there are present many of the same vessels and parts of vessels which were present in the first stage recorded. Some have been incorporated as parts of the larger vessels, others are still capillaries, while others have disappeared.

Considered as a whole, then, this series shows strikingly that arterioles and venules develop, at least in this region in tadpoles, not by a steady outgrowth of a single vessel, which grows straight ahead into a new region, giving off branches where they are needed, and fulfilling its predetermined destiny to grow in a particular place, but rather by the sending out of numerous capillaries, in various directions, which anastomose, adding new loops of circulating capillaries to those already present. Of these new loops some are so placed that a circulation is never established through them, and they disappear; others are incorporated as parts of arteriole or venule or remain as capillaries. The effect of the addition of new capillaries on the system already present depends upon the relation which the older parts bear to the new; thus a vessel which is at one stage the chief vessel of the region may entirely disappear, while another vessel, which is small, and has a slow circulation at one stage, may later be a

part of the main arteriole or venule of the region. It is impossible to predict at one stage, which way a capillary will go—whether it will increase in size, remain the same, or atrophy and disappear; it all depends upon the relation which it bears to the other vessels in existence at the time, and to those which are developed later. The endothelium is equipotential, then, and its differentiation into arteries, veins and capillaries is determined by factors outside the endothelial wall or in the lumen.

Further evidence for this view is found in the following experiments on chick embryos. They were performed to test another point, but the results are sufficiently interesting in their bearing on the problem of blood-vessel growth to deserve brief mention.

The anterior cardinal view of one side, from a point anterior to the otic vesicle to and including a part of the duct of Cuvier, was dissected out from chicks of two and one-half to three days incubation. The method employed is as follows: Berlin Blue is injected into the vein through a very fine glass cannula. As



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soon as the Berlin Blue mixes with the blood it forms a precipitate which plugs the vessel and sticks to the endothelial wall, outlining the position of the vein. Using this as a guide, the vessel is dissected out, with considerable of the surrounding tissue, to make certain that all is removed. The egg is then sealed and the chick allowed to develop further.

This experiment was performed successfully six times and in every case, there was found to be a large vein in the place of the one removed. In one case, the vein on the operated side was larger than the one on the unoperated side. The chicks were examined four to eight days after the operation.

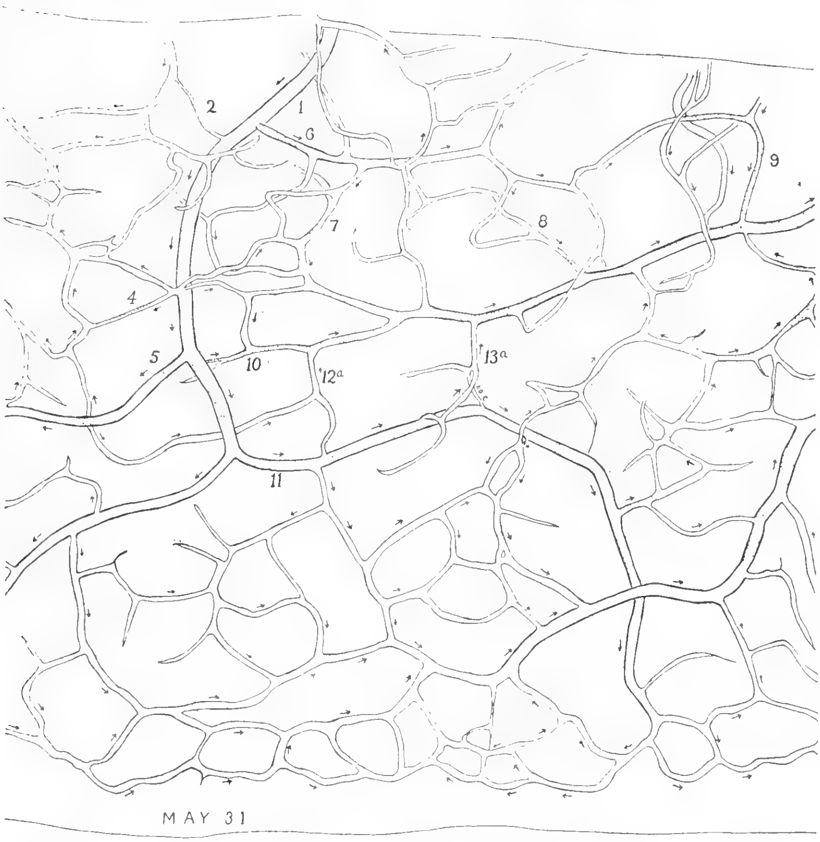
The conclusion seems justified that the secondary development of a large vein in the neck, in the place of the one removed, indicates that the mechanical conditions of the circulation favor the growth of a large vein in this region. Surely the new vein can hardly be considered as due to inheritance.

What are the laws which govern the growth of the endothelium, making the differentiation of such an elaborate system possible? What, in other words are the modes of reaction of blood-vascular endothelium?

THE FORMATION OF NEW CAPILLARIES

The first property to be noted is the capacity of the endothelium to send out sprouts. This process has been frequently observed in the transparent tails of living frog larvae, and verified by other studies, and is the generally accepted mode of spreading of the vascular system, after its primary differentiation. The sprout consists of an elevation of the endothelium which is sent out, usually starting at right angles to the vessel wall, and with a lumen continuous with the lumen of the parent vessel. The end of the sprout consists of a solid process of varying length, which may be in the form of a single thread, or of a thread with one or more branches. This process usually extends in a straight line from the parent vessel, for a varying distance, and may then curve. Sooner or later it reaches a similar sprout, or approaches a fully formed capillary, when it shows itself possessed of a prop-

erty most important for the development of a system of anastomosing vessels, namely, that blood-vascular endothelium has an affinity for blood-vascular endothelium, of such a nature that if two processes of blood-vascular endothelium draw near one another in their growth, a union will be formed between them ('cytotropism,' Roux). Equally important is the fact, readily observable in the tail of the frog larva, that blood-vessel endothelium avoids, in its growth, the cells of other tissues among which it grows, such as mesenchyme, and lymphatic endothelium. As a rule, the lumen eventually extends through the entire extent of this new sprout, it widens, and after a varying amount of time



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the circulation of blood cells commences, and a new circulating capillary has been added to the system. This whole process may, however, not be completed, for some sprouts grow out a short distance and are retracted, while some in which the lumen has been formed, never have a circulation, but retrogress—becoming solid, and disappearing. Throughout this process the endothelium remains complete, the lumen being separated from the tissue fluid outside by a complete investment of endothelium.

The facts concerning the morphological changes which take place in the formation of sprouts are clear enough; the question then arises as to why sprouts are sent out, to what sort of stimulus the endothelium responds when it sends out a sprout. The answer to this question is not entirely clear, yet certain facts together with certain general considerations justify the proposal of an hypothesis.

A study of the positions at which sprouts are formed and of the general direction taken in their growth shows that they are preceded in their formation by the growth of the other tissues and that they extend into regions where the amount of tissue not yet vascularized is greatest in amount. In the tad-pole's tail, at early stages, vessels develop first along the muscle—the thickest part of the tail. Later they grow out into the fin expansions, which attain a considerable size before vessels reach them. Growth of new capillaries continues in a general direction toward the dorsal and ventral margins of the fin, until eventually the plexus reaches nearly to the margin. During the growth of this first set of vessels, the fin remains thin, and the capillaries—save for the thickest part next the muscle—are all in a single plane. Later, the fin becomes much thicker and there occurs a corresponding new growth of capillaries, from the older parts of the plexus, which pass toward the epidermis, and form plexuses in two new planes.

In both cases it is clear that the growth of new blood-capillaries has been secondary to the growth of the outside tissue. It has been suggested by Thoma as an hypothesis that the stimulus responsible for sprout formation lies in an increase in blood-pressure. If this were so, one would expect to find them growing

out from the arterial rather than the venous end of the capillary, since, obviously, the pressure is higher in the arterial end. This, however, is not the case—at least, in the tad-pole's tail new sprouts grow out as frequently from the venous as from the arterial ends. Loeb ('93) suggests that the explanation of the new growth of blood-capillaries must be sought in the stimulus exerted by specific chemical substances outside the capillary. A similar suggestion is made by Evans ('09 B, note, p. 296), who says in discussing vascular and non-vascular areas in embryos: "We have to do here, perhaps, with a matter of cell chemistry or tropisins, for endothelium apparently avoids certain areas in the embryo—the non-vascular areas." In discussing this paper (see also '12, p. 584), Thoma ('11) argues that the findings of Evans fit in with his hypothesis, explaining nonvascular areas in the embryo as areas in which the pressure is high, due to the compactness of the tissues in such areas. As a result of this, according to Thoma, the difference in pressure between the blood inside the capillary and the fluid outside is less in such areas than in looser tissues where he supposes the pressure to be lower. There appears, however, to be a valid objection to the suggestion of Loeb and of Evans, which is found in the variations in the richness of the capillary plexus in the different organs and tissues of the adult. This is summarized as follows in Kölliker's Gewebelehre ('02, p. 670):

> Bestimmend für die Anordnung der Kapillaren ist die physiologische Leistung, und ergiebt sich als allgemeines Gesetz, dass, je grösser die Thätigkeit eines Organes, beziehe sie sich nun auf Bewegung oder Empfindung, auf Ausscheidung oder Aufsaugung, vor allem in den Lungen, der Schilddrüse, der Leber, den Nieren, dann in den Häuten und den Schleimhäuten, viel weiter in den Organen, die nur behufs ihrer Ernährung und zu keinen anderen Zwecken Blut erhalten, wie in den Muskeln, Nerven, Sinnesorganen, serosen Hauten, Sehnen und Knochen.

Thus we find that richness of capillary plexus may occur where the chief factor concerned is the passage of substances through the wall of the blood-capillary from the lumen outward, as in the kidney; again where the absorption of substances is apparently the chief factor, as in the intestine; and again, where removal

and absorption are approximately equal, as in the liver. It seems almost inconceivable that the great richness of capillaries in each of these cases can be due to the presence of an unusual amount of tropic substances.

The only common factor that can be discovered would seem to be the total amount of passage of substances through the endothelial wall, whether the direction be to or from the lumen of the capillary. That it is the quantity of substances passing through, and not some specific chemical body is strongly indicated by the fact that, in different organs, the substances which pass through the capillary wall are, in many cases, of a widely different nature.

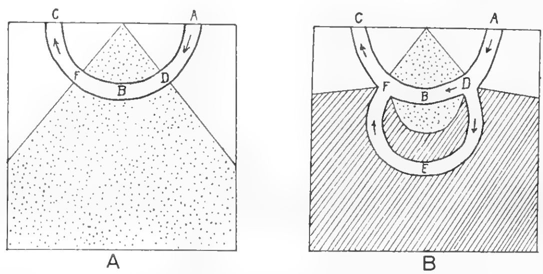

Fig. 9 Diagram to represent the modification in amount of tissue supplied by a capillary as the result of the addition of a new capillary. The dotted area indicates the area supplied by the portion of capillary *DBF;* the lined area that supplied by the new capillary *DEF.*

This, then, would seem to me to be the most likely hypothesis as to the nature of the stimulus which is chiefly responsible for the formation of new sprouts—that it is the total quantity of passage of substances through the endothelial wall. This conclusion is in agreement with that of Roux ('95) which has previously been fully quoted. According to this hypothesis, when the amount of fluid passing through any part of the endothelial wall exceeds a certain point, the endothelium reacts by sending out a sprout, which eventually becomes a new capillary, thereby increasing the endothelial surface and diminishing the relative amount of interchange through any part of the wall. Figure 9 has been constructed as a diagram to show how such a law, re-

duced to its simplest terms, would operate. The square (fig. 9 A) represents, diagrammatically, the amount of tissue supplied by the capillary *ABC*. The large area, supplied by the portion *DBF*, shown by stippling, causes the amount of interchange through the wall near *D* and *F* to become excessive, and new sprouts are sent out which form a new capillary, *DEF* (fig. 9 B). The relatively diminished area supplied by this new capillary is shown by cross hatching, while the greatly diminished area left for *DBF* is shown by stippling.

Let us see how this hypothesis accords with the observations of the present study. When capillaries first enter the fin expansion there is an extensive, growing non-vascular area, for the fin attains a considerable development before the blood-capillaries reach it. The formation of new sprouts at this stage is extremely rapid, a rapidity which may easily be accounted for by the excessive amount of interchange involved in the relatively enormous non-vascular area. The new formation takes place in one plane—the sprouts being sent out toward the dorsal and ventral margins of the fin, in the direction to and from which there is clearly the greatest amount of interchange, for the fin, at this stage, is quite thin. New sprout formation continues, in this plane, until the plexus of capillaries reaches nearly to the fin borders. As the borders are approached, sprout formation diminishes in rapidity, and this may be explained by the relatively smaller amount of tissue beyond the furthermost capillaries. Later, the tissue through the fin increases in all dimensions—in thickness, as well as in length and height, the capillaries increase in length, and a secondary formation of new sprouts takes place in the interstices of the old plexus. Many of the capillaries of this secondary set are in new planes, nearer the surface, especially in the thicker portions of the fin, near the muscle. It is significant that, in this secondary formation, new capillaries may grow out at places in the wall where vessels have been present but have been retracted, at an earlier stage. This secondary formation is best explained by the increase in exchange of substances due to the increase in amount of tissue.

It would seem difficult to explain the formation of new sprouts—especially the exact location at which they are sent out from the older capillaries—on the basis of the action of specific chemical substances. Such substances, if present, should act equally on all parts of the endothelium, resulting in streams of sprouts, sent out by each endothelial cell affected. We find, however, that excessive sprout formation occurs only in early stages, when the amount of tissue entirely non-vascular is very great. Later, when the tissue has received its primary supply, and when new sprouts are clearly associated with the general enlargement of the organs, the formation of new sprouts occurs in a much more orderly fashion, a single new sprout here, another there, a condition which seems much better explained by the hypothesis that the new formation is due to increase in interchange of substances beyond a certain point, than by supposing the presence of specific chemical substances.

On more general grounds, also, the proposed hypothesis seems the most plausible. It has been brought out especially by Roux that the growth, maintenance and atrophy of tissues is to a considerable extent regulated by the extent of their performance of certain functions in the body as a whole. Increased or diminished function results in increased or diminished growth. The endothelium of blood capillaries functions as a membrane through which substances pass to and fro between the lumen and the fluid outside. It would be in harmony with Roux' general conclusions, if it were found that the new growth, maintenance and atrophy of capillaries is regulated by the intensity of this passage of substances through their endothelium.

To be sure, it is impossible to go with certainty beyond the conclusions of Mall that, "with the new formation of tissue new blood-vessels may grow into it"—that it is "the growth of the tissue which leads the way," and that "into this new-formed tissue the capillaries grow." Nevertheless the proposed hypothesis as to the precise formative stimulus seems to the author to be more in accordance with the facts than the other hypotheses which have been suggested.

INCREASE IN SIZE OF CAPILLARIES TO FORM ARTERIOLES AND VENULES

It is obvious, in looking over the series of changes which take place in the capillaries, that, while some remain capillaries or are retracted, others gradually increase in size to form arterioles and venules. A study of the position of those which increase in size, shows that the increase takes place in the capillaries or parts of capillaries which are so placed that they form vessels for the supply or drainage of larger and larger capillary areas, so that their endothelium is subjected to the action of the passage of an increasing amount of blood. It would seem, then, that the conclusion is justified that increase in the size of the lumen of a capillary is regulated by the amount of blood flow. This is somewhat similar to the conclusion of Thoma, expressed in his first histomechanical law, that the size of the lumen depends upon the rate of the blood flow, at a minute distance from the wall. There is, however, this difference, that according to Thoma the rate of flow is the determining factor, while the present studies indicate that it is the total amount rather than the rate.

The capillaries in early stages—that is, during their early extension into the fin—are markedly wider than a few days later. At the same time the rate of flow in all vessels, at the earlier stages, is decidedly less than later—so that, coincidently with the increase in rate, there is, at this stage, a general diminution in the size of the lumen of all vessels. Moreover, in later stages, new capillaries are, for a time, relatively wide, with a slow circulation, and become narrower as the rate of circulation through them increases. Again, many instances may be seen, in any growing tad-pole's tail, of vessels remaining the same size or even diminishing in size until the lumen is obliterated, through which the rate of blood flow is relatively rapid, but, because of frequent complete stoppage of the flow, with the total amount very small, while, side by side with them, new capillaries, with a decidedly slower circulation, though they may diminish slightly in size, are not obliterated.

Another fact which must be referred to here is the well-known one that veins, in general, have larger diameters than arteries,

and yet the rate of circulation in veins is less than in arteries, while the amount of blood flowing through the two sets of vessels is the same.

It is clear, then, that the size of the lumen is not solely dependent upon either the rate or the amount of blood-flow. The chief difference in the condition existing in arteries as compared with veins, aside from the rate of blood flow, is the difference in blood-pressure. It would, therefore, seem that, with the same amount of blood to be propelled, the size of the lumen varies inversely as the pressure, providing the resistance is such that, with the greater pressure, there is a higher rate of blood-flow.

The diminution in caliber of blood-capillaries, after the early stage, is probably to be explained in this way. At the early stage the strength of the heart-beat is relatively small, as is shown by the slow rate of the circulation. Later the heart-beat evidently becomes stronger, for the rate of circulation increases markedly in all vessels. With this increase in rate there is diminution in caliber of all vessels.

If, however, we consider the changes which take place in a number of vessels which are subjected to approximately the same pressure conditions and rate of circulation, we find that the lumen varies with the amount of blood flowing through. Given a sufficient amount of blood to fill all vessels, and a sufficient strength of heart-beat to keep the capillary circulation up to the necessary standard, and it is found that the size of the vessel varies with the amount of blood flowing through. Thus, of two capillaries near one another, the one so placed that it forms a pathway for the supply or drainage of an enlarged capillary area has an increased circulation and increases in caliber, while the one not so situated remains the same size or becomes smaller.

The objection might be raised that the movement of the blood is not a formative factor—that the vessel merely fits the stream, and that its size is solely the result of the mechanical distention. That this is not valid is shown by the fact that vessels in which the circulation ceases altogether, or in which the circulation never starts, grow smaller and smaller until their lumen is entirely

obliterated. (This will be taken up more fully later.) Thoma has made similar observations on ligated vessels—finding that, in spite of the mechanical distention due to blood-pressure, the lumen of ligated vessels becomes reduced to zero between the point where the last branch is given off and the point of ligature.

We are, then, in agreement with Thoma, on this point, that the endothelium responds to the action of a moving fluid. To us, however, it appears that Thoma's claim that it is the rate of flow is not justified. Rather, it appears that it is the amount of blood flow through a vessel which determines the size of its lumen. To be sure the factor of rate of flow, as well as the closely related factor of blood pressure, cannot be disregarded. To a certain extent, however, their action appears to be the opposite of that claimed by Thoma, for with increased rate there may be a diminution in size.

THE REGRESSION OF VESSELS

It has already been mentioned that in the growth of blood vessels many capillaries and parts of capillaries disappear. This process was referred to briefly in a former paper (2), '09 and will now be examined more in detail. On the series of records shown, there are many instances of the disappearance of vessels, in some cases of vessels which had never attained a circulation, in others of vessels which had had an active circulation.

First let us see what are the morphological changes which take place in the 'disappearance' of a vessel. The process has been watched carefully many times, and two sets of records are reproduced to show the details (figs. 10 and 11). The first change to be noted is a narrowing of the lumen. Next there appears an interruption of the lumen by the formation of a solid portion. The solid part may start near the middle of the capillary or nearer one end, and it gradually increases in extent toward the two ends. As it increases, the solid portion becomes narrower and narrower, until a varying amount of the former capillary is represented by only a fine thread. This thread becomes thinner until it is barely visible, and eventually disappears, leaving the two ends forming blind-ending projections from the

vessels to which the former capillary was connected. These remains of the capillary shorten by the retraction of the endothelium into the vessels with which they are connected, until they form only a slight swelling on the surface. This eventually disappears, and there is nothing left to mark the site of the former capillary. In figure 11 may be seen the movement back into the connecting vessel of a nucleus and a small pigment granule, from the capillary which is undergoing retrogression.

In this process of retrogression of blood-vessels as it is seen in the living animal, there is nothing that even remotely suggests the

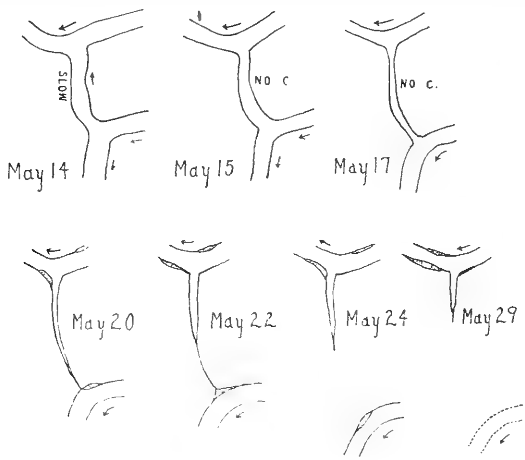

Fig. 10 Several stages in the narrowing and retracting of a vessel in the tad-pole's tail (rana palustris). *NO C.*, no circulation.

transformation of an endothelial cell into a mesenchyme cell, or of any other type of cell. The impression is gained that the entire protoplasm is withdrawn into the parts of the system which persist, and that none of it is lost. Certainly some of it is withdrawn, as for example, in the case of the nucleus mentioned above. If any part fails to be withdrawn, it must be that it is dissolved, for the last that can be seen is a thread so minute that it is barely visible with high power lenses.

In the tail of the frog larva parts of blood-vessels rarely become completely isolated from the rest of the system, as has been de-

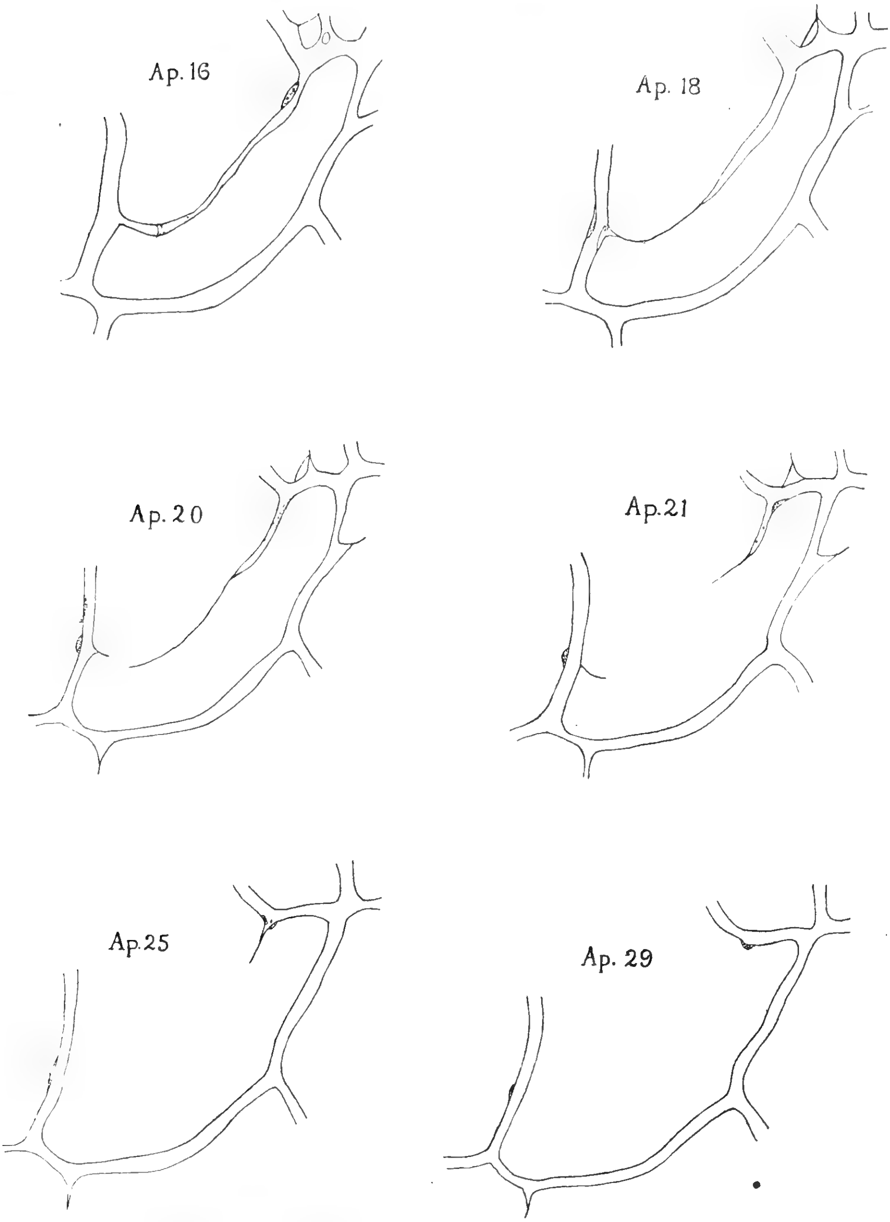

Fig. 11. Stages in the retraction of a capillary in the tail fin of a rana pipiens larva.

scribed for vessels elsewhere. In order to observe the fate of such a capillary I completely isolated a small section of a capillary by cutting through its connections. Much to my surprise, this isolated capillary was found, two days later, to have formed an anastomosis with one of the circulating capillaries (figs. 12). Thus a capillary which has become isolated, does not lose its blood-vascular-endothelial properties, and may be reincorporated in the vascular system. It is perhaps worth noting that this vessel was in a region, near the margin of the fin, where active new formation of vessels was taking place.

What, then, are the factors which lead to the regression of capillaries? In keeping a record of the developing vessels, a record was also kept of the presence or absence of circulation in each capillary, and of the approximate rate of the circulation. On looking over the previous records of the circulatory conditions of any vessel which has undergone retrogression it is found that this process is preceded by a period in which the circulation has ceased. This period is usually preceded in turn, by a period in which the circulation has diminished in quantity. This latter, of course, applies only to the vessels in which a circulation has been established, for, as has been said, some vessels are withdrawn before any circulation has been established in them. Thus many definite records have been obtained in which the retrogression of a capillary has been associated with the stoppage or absence of circulation. The conclusion, therefore, seems justified that a vessel which is connected with the rest of the circulating system of vessels, retrogresses and disappears if the circulation within it ceases for a sufficient length of time.

The finding that the diminution in size of lumen which precedes the retraction of capillaries involves the property, on the part of the endothelium, of reacting to the amount of blood flowing through the vessel, agrees, in part, with Thoma's first histomechanical law. According to this law, however, it is the rate ('Geschwindigkeit') of blood flow which is the determining factor ('93, p. 37 ff). In these studies it appears that, in capillaries, at least, it is not the rate but the amount of blood flow which is

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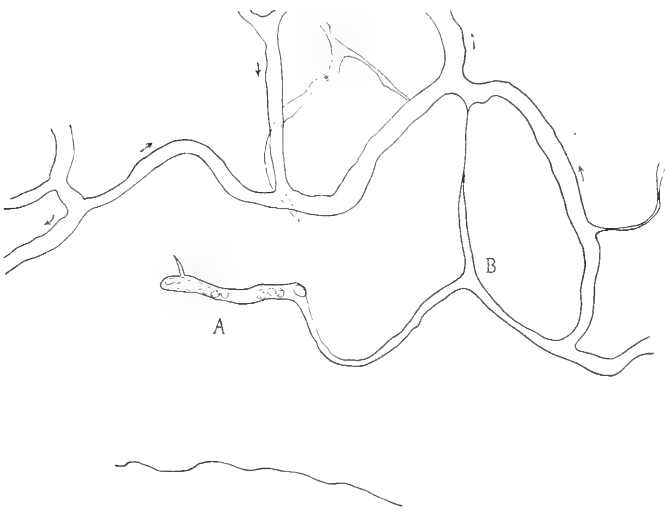

12 *b*

Fig. 12 Drawing to illustrate fate of an isolated portion of blood-vessel. The portion A was cut through at the two points labeled *X* in drawing a. Two days later (b) this isolated portion had formed a connection with the rest of the blood-vascular system at B.

important. For while in some capillaries there is diminution in rate before retraction, others become smaller and have the lumen reduced to zero through which the rate never has become slower. The only common factor between these two is the total amount of blood flowing through. In the second type this is shown by the fact that though the flow is rapid, it is scanty and intermittent.

The question naturally arises why the circulation becomes slower and even ceases altogether in certain capillaries, in a region where an active new growth of capillaries is taking place. In the case of many of the capillaries, the answer is pretty clear. With the continuous formation of new vessels, the pressure conditions in the vessels already present are changed, and a capillary which formerly may have been the only vessel between vein and artery, may later form merely a cross connection between two parallel vessels, in which the pressure is equal. With an equal pressure in the two ends of the capillary, the flow of blood through the capillary ceases. The majority of the cases are evidently of this character, as may be seen by looking over the records (cf. branches 12 and 16, figs. 1 to 5). There are, however, other vessels to which this does not seem to apply, particularly branches of the larger arterioles (cf. branch 3, figs. 1 to 6). A considerable percentage of the branches of each vessel which differentiates into an arteriole disappear. In the case of these capillaries the retraction is preceded by a period during which there is no flow through the vessel, but the period of absence of circulation is not preceded by a slowing of the circulation. Instead of a slowing there is a diminution in the amount of blood passing through, while the rate remains rapid. Periods of total absence of circulation alternate with periods in which a few cells pass through at a rapid rate. In this condition the capillary branch affected leaves the arteriole at a right angle, and is relatively small as compared with the arteriole. Frequently the entrance to the branch becomes plugged by an erythrocyte or a leucocyte, which causes a stoppage of the circulation. The retrogression of such vessels takes place usually much later than in the case of the other retrogressing vessels, such a vessel often remaining for several days, with a gradual increase in the

length of the periods of no circulation before retrogression takes place. I have no entirely satisfactory explanation to offer to account for the stoppage of circulation in these branches. It is possible that the increasing thickness and elasticity of the arteriole causes a constriction about the opening of the branch, until it becomes so small that a blood cell cannot pass through. Another possibility which has suggested itself is that the narrowing is due to a suction on the branch caused by the rapid passage of fluid past an opening into a branch going off at a right angle. Whatever the explanation, however, the important fact remains that the retrogression of these branches is associated with a diminution in the total amount of blood which passes through them.

There remain vessels in which the blood-flow diminishes and the lumen decreases, with resultant retraction, for which none of the factors thus far suggested seems to offer a satisfactory explanation. It is quite a striking fact that, in most new capillaries the blood flow is at first slow—usually slower than in older capillaries—that the circulation through the newer capillaries increases while that through some of the older ones diminishes. It would seem that here, as in the case of the formation of new sprouts, a regulating factor must be looked for in the rate of interchange of substances through the wall. A glance at the diagram (fig. 9) will illustrate the significance of this suggestion. While the portion of the capillary *DBF*, in figure 9 A, is so placed that it forms the medium of interchange for the large stippled area; in figure 9 B, the new capillary *DEF*, which has developed peripherally, has taken over the greater portion of this area, leaving only the small stippled area for capillary *DBF*. It seems logical to suppose that, if the area supplied by such a capillary is sufficiently reduced so that the amount of interchange through the wall falls below a certain point, the capillary will diminish in caliber and eventually retract.

The capillary is concerned chiefly with this interchange of substance, and it is difficult to escape the conclusion that the growth processes of endothelium are regulated by it. Were they not, it seems impossible to conceive of how an organ becomes

sufficiently vascularized and how supernumerary capillaries are disposed of—in fact, how any equilibrium is established between the extent of metabolism of the various organs and the richness of their blood capillary supply.

The precise nature of the stimulus may well be conceived as a physical one—the friction produced by the passing through the endothelium of fluid substances, just as the other regulating factor—the blood-flow over the interior of the endothelium—is a physical character. With a certain (undetermined) amount of interchange, the endothelial cell remains unchanged; with increased interchange beyond a certain (undetermined) point, the endothelial cell sends out a sprout; with diminished interchange below a certain (undetermined) point the endothelial cells constrict the lumen and are eventually withdrawn into the active capillaries, leaving no trace of the capillary which had fallen into disuse. It is probable, if this hypothesis is correct, that the retraction of capillaries through which circulation has ceased as the result of equalized pressures at the extremities or of plugging of the vessel, is due to the operation of this factor of endothelial response to diminished interchange.

These two factors, then, amount of interchange through and amount of flow over the inner surface of the endothelial wall of capillaries appear to be the chief ones concerned in the regulation of the new growth of capillaries, their maintenance, increase in diameter to form arterioles and venules, or decrease in diameter with eventual solidification and retraction. Of these two factors, the amount of interchange is primary and the amount of flow secondary, since increased or diminished blood-flow depends in the main upon changes incident to the formation of new capillaries.

Endothelium subjected to either of these factors will survive, grow, or retract according to the intensity of the stimulus. If the blood-flow is increased, there is increase in the diameter of the vessel. If the rate of interchange is increased there results sprout formation. Diminution of blood-flow causes diminution in diameter, and diminution of interchange, narrowing, solidification and retraction of endothelium.

Fig. 13 From series shown in figures 1 to 8, showing vessels of stage of May 31 (dotted lines) superimposed on vessels of stage of April 15 (solid lines). The corresponding vessels are lettered. Enlargement the same for both stages.

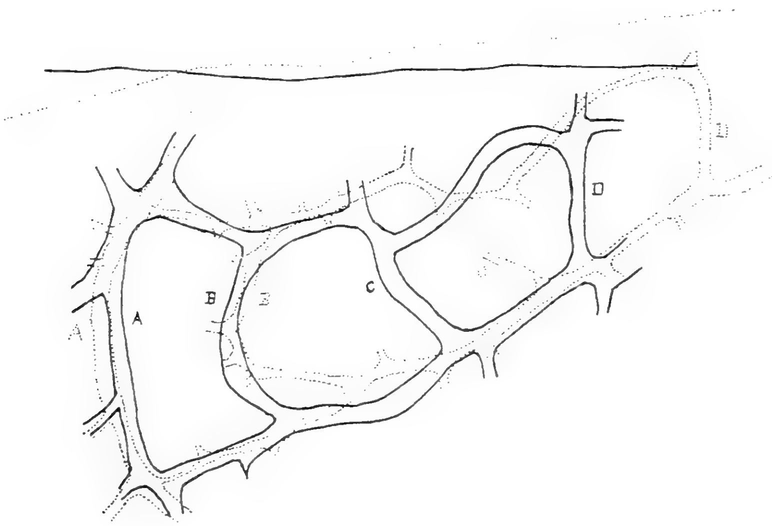

Fig. 14 Same vessels as shown in figure 13. The vessels from stage of May 31 (dotted lines) have been reduced, by means of the pantograph, until they have the same dorso-ventral length as vessels from stage of April 15 (solid lines).

THE INCREASE IN THE LENGTH OF VESSELS

With the general growth expansion of the tissue, the question arises as to what effect this has on the blood vessels present in the midst of the organ. In figures 13 and 14 there have been superimposed capillaries and vessels which were present at the earliest and at the latest records. In one case (fig. 13), they are both drawn at the same degree of enlargement. In the other (fig. 14) the drawing of the older stage has been reduced, by means of the pantograph, enough so that the two sets of vessels have the same dorso-ventral dimensions. Measurements show that the increase has been greater in the antero-posterior than in the dorso-ventral direction. The increase in the dorso-ventral measurement of the fin expansion has been in the proportion of 1 to 2.22; while in the antero-posterior measurement (the total length of he tadpole) the increase has been in the ratio of 1 to 2.73. Thus the ratio of the dorso-ventral to the antero-posterior increase is about 7:10. Almost the same proportion is found to exist between the measurements of corresponding parts of the capillary plexus at the same two stages. Thus the ratios obtained are, for the dorso-ventral increase: 1. to 2.2, for the antero-posterior increase: 1. to 2.9. It is possible that the agreement between the two sets of antero-posterior measurements would be even closer, had the measurement been made of the increase in length of the tail, instead of the increase in the length of the entire larva, including the head. It is obvious that the growth of the tissue has caused a proportionate growth in the length of the blood capillaries, and the size of the capillary mesh-work.

There would seem to be but one possibility as to the factors responsible for this increase in length of vessels, namely, the one proposed by Thoma ('11), as his second histomechanical law, that increase in length of vessels results from a tension exerted in a longitudinal direction on the vessel wall, by the surrounding tissue. In the tail of the frog larva, the space between the blood capillaries is occupied mainly by branched mesenchyme cells, from which fine fibrillae in great abundance are given off in all direction, surrounding and supporting the blood-capillaries, lym

phatics and nerves, and extending between the right and left layers of epidermis. As the tail grows there is an increase in the number of these cells, and in the size and complexity of their processes. It is clear that such an increase in the tissue filling up the space formed by a blood-capillary mesh-work, the fine processes of the tissue being in contact with the blood-vessels, would lead to a pushing and pulling on the capillary wall, and it seems quite safe to infer, as Thoma has done, that the growth in length of vessels is due to the response of the vessel wall to this mechanical tension.

CHANGE IN THE ANGLE OF BRANCHING

It was somewhat surprising to find how closely the direction and pattern of the capillaries which persisted resembled, in the latest stage, their pattern in the earliest stage. Thus, bends, present in the capillary at its first formation, although they may diminish, or disappear entirely, may be retained throughout. To a considerable extent, also, the angles between branches remained nearly the same as when first formed. It is apparent that the capillaries, once formed, are held fairly rigidly by the surrounding network of fine connective tissue fibrillae. There is, however, a marked change in the angle of branching, in the case of the arterioles. Here one sees the working out, in a general way, of the laws of branching discovered by Roux, for the smaller the branch, relative to the main stem, the nearer its angle of branching approaches a right angle, and the larger the branch, the more acute—relatively—its angle of branching. Figure 15 illustrates the change. On March 18, the two branches—B and C—into which A divides are approximately equal, and the angle of branching of each is nearly 90°. This stage is an early one—the capillaries are newly formed, and are characteristically wide, and the angles are those which happened to form as a result of the direction taken by the new sprouts. March 20, branch B is slightly larger than C, and there is a tendency for its angle of branching to become slightly more acute. March 24, April 13, and April 26, however, show a progressive increase in the size of branch C over branch B, and a corresponding dimi-

nution of its angle of branching, as compared with that of branch B, until, April 26, it forms, as it leaves the main stem A, almost a direct continuation of A, while the angle formed by B is much nearer a right angle. It will be noted that, in the later stages, there is a reduction in the size of all the vessels. With this reduction, however, there is marked increase in rate of flow. Other examples may be seen by comparing, in the successive stages of the main series shown, the branches from the chief arteriole.

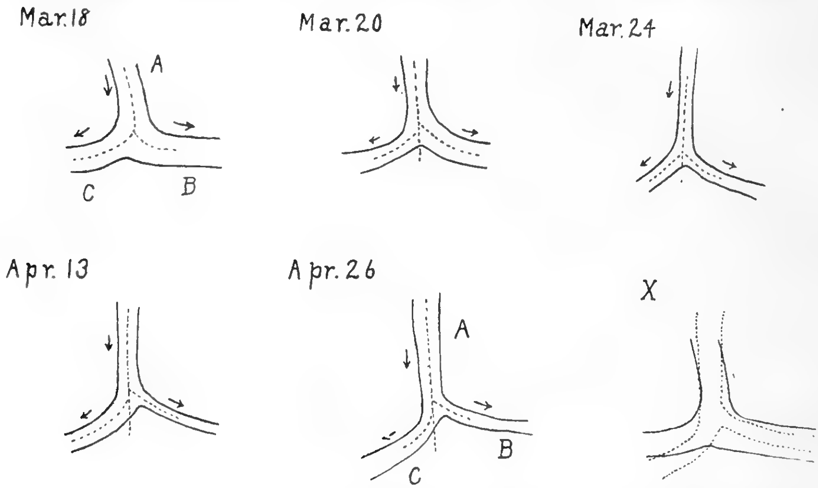

Fig. 15 Several stages of blood-vessels in the tail of rana sylvatica larva, to show changes in angle of branching. In x, stage of April 26 is shown in dotted lines, superimposed on stage of March 18.

In these cases, the angle of branching is found to vary according to the relative size of the branch—the larger the branch the smaller the deviation from the line continuing the axis of main stem, while the smaller the branch, the larger the angle—until, when the disproportion is great, the angle reaches 90°.

These results corroborate the results of Roux' studies, which were based on measurements of arterial branches in adult animals. Roux stated his results in the form of a law, namely, that the size of the branch divided by the size of the main stem gives a series of figures which vary about as the cotangent of the angle of branching. He considered that the size of the angle represents

the response of the tissue to hydrodynamical factors, and that the angle formed is always the one by which the minimum of friction is permitted.

The same problem is discussed by Thoma, ('11, p. 26) who agrees in the main, with Roux' findings, but considers that the blood-vessel, in assuming this shape, is merely responding to the rate of blood-flow, according to his first histomechanical law.

It would seem that Thoma's explanation (if amount of flow is substituted for rate) is sufficient, for, if fluid flowing through any tube tends to leave at a greater and greater angle the smaller the opening, then, if the blood vessel did not correspond with the direction naturally taken by the fluid stream, part of its wall would be subjected to the action of a greater flow of blood, than other parts, and would enlarge, while other parts would retract from the opposite cause. By this, the branch would be remodelled until its angle of branching represented that in which the blood flowed in even amounts over all parts of the wall.

My observations on the angle of branching add nothing new to the results obtained by Roux, except that they represent studies on the same vessels at different stages, and give a picture of the actual changes in shape going on, hand in hand with the changes in the relative sizes of the branches, together with an approximate record of the relative amount of blood flow.

STUDY OF VESSELS IN TAD-POLES WITHOUT HEARTS

Since it has been found that a considerable development takes place in embryos deprived of a circulation by eliminating the heart-beat, it would seem, at first sight, that an opportunity offered itself here to test certain of the hypotheses dealt with in this paper—to find out whether any, and, if any, how much development of arteries, veins and capillaries takes place—particularly in the portion of the tad-pole's tail studied.

Brief reference has been made to such studies, and they will now be referred to more fully.

Roux ('95, p. 83) refers to a picture given by Dareste ('77, Pl. VII, fig. 6) of a chick embryo in which the embryo proper failed to develop. In commenting, Roux says:

> Nach dieser Abbildung Dareste's und einigen von mir zufällig aufgefundenen, weit ausgebildeten Fällen treten in diesem Capillarnetz schon einige den normalen grösseren Gefässen entsprechende Richtigungen deutlich hervor, wie ich sehe, die entsprechende Erweiterung derselben und die Verdickung ihrer Wandung fehlt; der Sinus terminalis ist jedoch ausgebildet und beweist so allein schon die vererbte localisierte Anlage eines typischen Gefässes.

Embryos without circulation were produced by J. Loeb ('93) who found that, by growing embryos of fundulus, a salt water minnow, in the proper concentration of potassium chloride, the beat of the heart could be entirely eliminated, or so diminished that all circulation of the blood was absent. The development of the embryo, however, continued for several days—about half the normal hatching time. In such embryos Loeb found an extensive development of blood-vessels which, except for irregularities in caliber, resembled in distribution the vessels in normal embryos. He concluded that:

> Die mechanischen Ursachen für das Wachstum der Gefässwände sind deshalb nicht im Gefässlumen zu suchen, sondern in allen oder einzelnen Zellen der Gefässwände und die Abgabe von Aesten ist bestimmt durch innere Ursachen in den Zellen der Gefässwände oder durch Reizursachen, die von der Umgebung ausgehend, diese Zellen treffen, ähnlich wie im Falle der Stolonenbildung von Hydroidpolypen.

Patterson ('09, pp. 87–88) studied the area vasculosa of chick embryos in which the development of the embryo proper had been prevented by operation upon the unincubated blastoderm. He describes the finding of vessels which radiate toward the remains of the embryo, and which he interprets as omphalomesenteric arteries.

Knower ('07) studied the development of frog larvae from which the heart anlage had been removed before pulsation had started. In a brief summary he states that—"the aorta, the large veins and the segmental vessels are laid down." "Both arteries and veins are very abnormal and have a few well-defined branches. All vessels become much distended and follow very irregular courses." He found no vessels in the fin expansions of the tail.

Stockard found in fundulus embryos, in which the circulation of blood was inhibited from the start by the use of alcohol, that the vessels of the yolk sac and many of the vessels of the embryo, including the two aortae, are formed, that some of them become much distended, and that they may persist without circulation for many days. While he gives no detailed or careful study of the exact amount of development of the vascular system, or the amount of retraction of vessels, he states ('15, B, p. 586) with reference to the aorta:

> The aorta in old embryos that never had their blood to circulate and in which the heart is actually a solid stream of tissue, grows and attains a well-developed lumen and a wall lined with endothelium and surrounded by concentric fibers of connective tissue, as is shown in figure 4 a in the previous paper, drawn from such a specimen. This vessel is very slow to degenerate, in fact, it shows no sign of degeneration and actually persists as long as the embryo is able to exist without a circulation, for 30 days or more." . . . . *The function of the vessel as a blood conductor, therefore,* seems in these embryos of Fundulus, *to have little if anything to do with its early development and not much effect on its ability to survive.* . . . . These facts are most significant in a consideration of the influence of function on growth and development, auto-differentiation. Here it is seen that the structure both grows and develops in entire absence of function.

The descriptions of the extent of vascular development which takes place without a blood circulation, as given by these investigators, while meagre and incomplete, agree in the finding of an extensive vascular system, in which at least some of the main vessels appear to have developed sufficiently to give the impression of being fairly similar to the vessels in normal embryos. Thoma ('93, p. 28) recognized, in chick embryos, that there is not only an extensive development of capillaries in the extra-embryonic area, but part of the aorta is well developed before the heart beat commences.

He offers two possible explanations of the early development of the aorta. One is that there is an inheritance of an anatomical structure which is in agreement with the structure resulting from the action of mechanical forces. He says (p. 28):

> Man kann somit nur feststellen, dass die vererbte Form sich in Uebereinstimmung befindet mit jenem allgemeinen von mir aufgestell-

ten Gesetze, welches das Wachstum der Gefässwand von den Strömungsverhältnissen des Blutes abhängig erscheinen lässt.

At another place, however, (p. 32) he suggests that the development of large vessels in the site of the two primitive aortae may be due to favorable mechanical or nutritional conditions.

That other main vessels develop, in chick embryos, before circulation commences, has been shown by Miss Sabin ('17) and referred to earlier in this paper.

In order to study the character of the blood-vessels in the tail fin of frog embryos deprived of a circulation, the type of plexus formed, and the mode of growth, I have employed Knower's method on frog larvae, and studied the vessels in the fin expansion of the tail. The heart was removed after it was sufficiently developed to be clearly visible, under the binocular microscope, but before it had started to beat, by making an opening through the skin, into the pericardial cavity grasping the heart with a pair of forceps, and dissecting it loose with a needle. In some cases a small pulsating fragment was left but there was no blood-circulation. Embryos operated on in this way rarely live more than seven days, if the weather is warm, though they survive for ten to twelve days in cool weather. As Knower had described them, they become greatly swollen, due to the accumulation of fluid in the body cavities. They are very active, swim about the dish restlessly, and respond quickly to stimulation.

Unlike Knower, I found a considerable vascular development in the tail fins. Since the tail is opaque in early stages, due to the yolk and pigment, it was not possible to obtain very striking records of the growing blood-vessels. In several cases, however, the tail became sufficiently clear to permit records to be made. In one case records were made covering three days of growth of the vessels in the dorsal fin, and a considerable amount of growth was observed. The blood-vessels in the dorsal fin, in these larvae without hearts, form a primitive close-meshed plexus, of delicate vessels. In some cases the vessels are distended with blood cells, in others they are distended with a clear fluid, while in others they are very narrow. The blood cells are pushed about in the vessels by the movements of the embryos, and are

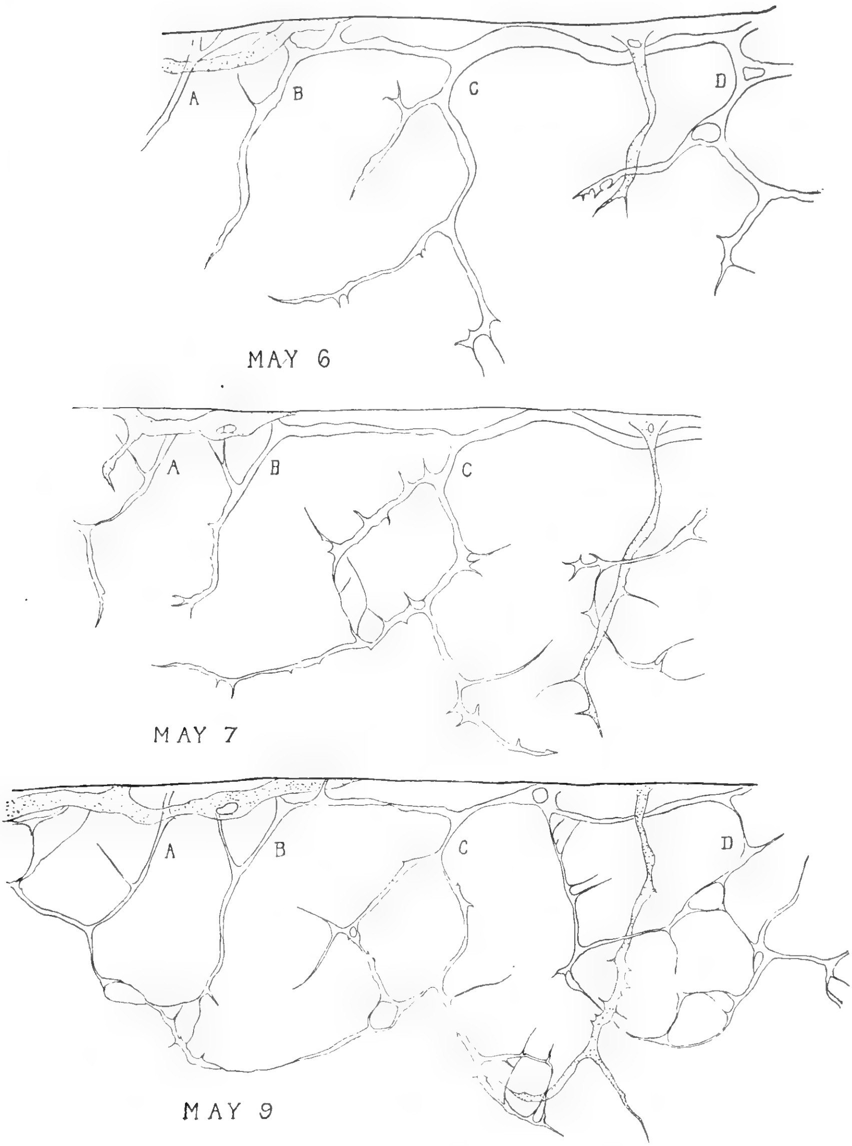

Fig. 16 Vessels from a portion of the tail of Rana Pipiens larva from which the heart was removed on April 27, before pulsations had started. Drawings made May 6, 7 and 9 of same region of dorsal fin. *A, B, C, D*—indicate the same blood-vessels. Lymphatics are dotted.

also moved about to some extent by the action of gravity. Thus, vessels which have been empty, have a little later been found to be packed with cells, and vessels filled with cells, may later be quite empty, or filled only with a clear fluid. Figure 16 shows some of the vessels in the dorsal fin of such a larva, on three different days.

On comparing the three records, it is obvious that growth has taken place by the formation of sprouts, which are at first narrow threads, but which later acquire a lumen. Anastomoses form between neighboring sprouts, and no additions are made by outside cells. In fact the vascular plexus extends in essentially the same manner as in the normal tail. There is, however, a larger number than in the normal, of fine solid processes. In this particular specimen the vessels are very narrow, and contain no blood cells.

It is to be noted that the vessels, once formed, show no tendency to differentiate further—into arterioles or venules. All diminish somewhat in caliber. In other specimens all the vessels are considerably distended. While three days is not a sufficient time, even in the embryo with a circulation, for much differentiation, still, in stages as early as this, at least a beginning differentiation is noticeable, as may be seen in the series shown in figures 1 to 3.

It is of interest to note the growth of lymphatics in the embryos deprived of blood circulation. Knower's observation that the anterior lymph hearts in such embryos are larger and beat more strongly than in normal embryos, was confirmed. In the tail fin, lymphatics grow out often beyond the bloodvessels, although in the normal embryos at this stage and in this species (Rana pipiens) the blood-vessels in this region grow out well in advance of the lymphatics. The lymphatics are somewhat wider than in normal embryos. The mode of growth of lymphatics is the same as in normal embryos, by the extension outward of sprouts, and there is no tendency for the lymphatic and blood-vascular endothelium to form anastomoses with one another.

The enlarged caliber of the lymphatics is of interest, especially in connection with the enlarged lymph hearts, and with the ob-

servation made by Knower, and confirmed by myself, that there is movement of lymph, as shown by the passage through the lymph heart of an occasional blood-cell, for it shows that passage of lymph into lymphatics is not dependent upon the maintenance of a certain amount of blood pressure.

The studies on embryos without circulation show that without the action of the mechanical factors concerned with circulation an extensive development of blood-vessels takes place; that some vessels—at least the aorta in chick and fundulus embryos—differentiate beyond the capillary stage. My finding that, in such embryos, growth may occur by the usual process of sprouting, indicates that this property of endothelium is not dependent for its early manifestation upon the action of the specific mechanical or chemical factors which seem to regulate it in later stages. So far as they bear on the main problem of this investigation, these results are important as indicating the extent and character of development of the vascular system which may take place without the mechanical factors concerned with the circulation of blood. It has been clearly brought out, especially by Roux, that there are two chief stages in the development of each organ or tissue. The first stage is the stage of 'auto-differentiation,' in which, by virtue of what our ignorance forces us to call heredity, or as Noel Paton expresses it, 'hereditary inertia,' each tissue differentiates and develops to a certain point. The second stage is the stage of 'functionelle anpassung,' functional adaptation, in which the further growth is regulated mainly by factors concerned in a quantitative way with the especial function of the organ or tissue. It is, therefore, consonant with our knowledge of many other organs and tissues to find that blood-vessel endothelium differentiates and grows for a certain period, and even that a vessel such as the aorta develops, as the results of 'heredity,' and without the action of mechanical forces. Such a finding affords no objection to the thesis that, in their later growth, blood-vessels are subject to the regulative action of the moving blood stream, the blood-pressure, the mechanical tension exerted on the wall by outside tissues, and the amount of passage of substances through the vessel wall. In fact, were it

not true of blood-vessel, as it is of other tissues, that two such phases exist, the vascular system would furnish a marked exception—at least, so far as our present knowledge of other tissues and organs goes. The question as to how far the vascular system might develop without heart-beat has not yet been satisfactorily worked out and presented.

SUMMARY AND DISCUSSION

The results of these studies on living blood-vessels are:

a. An extensive vascular development takes place, in the early embryonic stages, which is independent of the mechanical factors concerned with the circulation of the blood and the interchange of substances through the endothelial wall. During this stage, which to some extent precedes the inauguration of cardiac pulsation, it has been found that the aorta develops beyond the capillary stage (Thoma ('93)) and that a number of other main arteries and veins are formed (Miss Sabin, '17) while several observers, by producing embryos with the heart beat eliminated, have found, apparently, that a number of the other main vessels develop. My own studies on frog embryos without hearts, show that extension of the blood-vascular system during this primary stage takes place by sprouting, and by the formation of anastomoses between sprouts.

Thus the vascular system, like other systems about which we have knowledge, differentiates and is carried a considerable distance on its developmental course—manifesting the property of extension by sprout formation, and forming some of the larger arteries and veins—as a result of 'hereditary inertia,' or 'self development.'

This stage, however, comes to an end relatively early; and the vascular system, for its further development into the complicated and nicely balanced system of the adult animal comes to be dependent upon the mechanical factors concerned with the pull and push of outside tissues, with blood-pressure and blood-circulation, and with the interchange of substances through the wall. The picture presented in the tails of a-cardiac tad-poles

by the irregular indifferent plexus with no tendency toward transformation into arterioles, venules and capillaries, showing little more than the ability to send out and withdraw sprouts, is in marked contrast to the picture presented by the vessels in the same region, in tad-poles with a healthy circulation.

b. In normally developing tad-poles, the establishment of circulation brings into play, on the endothelium, the distending action of the blood-pressure, the mechanical friction of the moving blood stream, the mechanical (and possibly chemical) action produced by the passage of substances through the endothelial wall, in the interchange between the blood and the tissue fluid, and the pull and push of the enlarging and shifting outside tissues and organs. These studies, which have been principally confined to this stage, indicate, in general agreement with Thoma, Roux, Mall and Evans, that the new factors come to play a predominant part in the regulation of the growth of the vascular system, a part so important that the vascular endothelium may be said to depend for its growth on its response to the action of these forces.

The morphological changes are as follows:

The blood-vascular system extends by the well-known method of sprout formation. Blood-vascular endothelium has an affinity for other blood-vascular endothelium, cytotropism, (Roux), and avoids cells of other types of tissue, so that connections form between one sprout and another, or between a sprout and a fully formed capillary, while no connections form between a sprout and foreign cells. Thus the vascular system grows as a continuous network.

A sprout once formed may have one of several fates; it may enlarge to form part of an arteriole or venule, which may become an artery or vein, or it may remain a capillary, or it may retrogress, losing its lumen, separating in the middle, and retracting into the capillaries with which it is connected at its two ends.

Changes take place in the angle of branching, and vessels increase in length.

The mechanical conditions which regulate these morphological changes are the following:

The new formation, enlargement, maintenance and atrophy of capillaries is dependent upon two factors (1) the amount of blood flow and (2) the amount of interchange of substances through the wall.

1. A capillary or part of a capillary so located that an increased amount of blood passes through it, increases in diameter until it may form part of an arteriole or venule; one so located that there is no especial increase or decrease in the amount of blood passing through remains a capillary; while to decrease or absence of blood-flow the capillary reacts by a diminution in lumen to final solidification and complete retraction. This, in a general way, agrees with Thoma's first histomechanical law, according to which the diameter of the vessel responds to the action of the moving blood stream. A difference, however, lies in this, that, while Thoma assigns chief importance to the rate of the blood stream, I find, in capillaries, that it is rather the amount of blood flow.

The changes in the amount of flow through different capillaries are brought about in various ways. The addition of new capillaries beyond, may cause an increase in a capillary so placed as to help supply or drain the new area. Again, the opening up of a new capillary may place an older capillary in such a position that it forms merely a cross connection between two parallel vessels, in which the pressure is equal, bringing about a slowing or stoppage of circulation in the older capillary. In certain cases of stoppage of circulation the cause is more puzzling—that is, in case of branches of capillaries which enlarge to form arterioles. It is suggested that the stoppage, here, is due to the constriction about the beginning of the branch, resulting from the increased thickness and elasticity of the arteriole. Some support is lent to this explanation by the fact that blood cells are often seen to plug the entrance to such branches often for long periods.

It is possible, however, that the narrowing of such vessels, as well as the slowing of circulation in the case of other capillaries, is due to the second factor mentioned.

2. The amount of interchange through the wall. This is pro-

posed as an hypothesis, because it seems to fit the facts better than any other. According to this hypothesis, the endothelium of blood-capillaries responds to the passage through it of various substances, in the interchange which takes place between the blood and the outside tissues. To an increase, beyond a certain maximum, the endothelium is thought to react by sending out a sprout; to a diminution, beyond a certain minimum, in a capillary which is not so placed as to form part of an arteriole or venule, the capillary is thought to react by narrowing its lumen; while for the maintenance of a capillary, a certain intensity of interchange is thought to be necessary.

The formulation of this hypothesis, which agrees, in general with Roux' conception, is merely carrying the explanation for the new formation of capillaries a step further than Mall, who recognized, as did Thoma, that the ultimate cause for new growth of capillaries lies in the growth and metabolism of outside tissues. It seems to fit more facts than the suggestion of Loeb and Evans that the cause lies in the action of specific substances outside the capillaries, or Thoma's hypothesis that it results from increase in capillary blood-pressure. It needs, however, further proof, before it can be accepted as a "law of growth."

The changes in the angle of branching, which were observed, represent a response to the relative amount of blood-flow through the branch, as compared with the main stem. If a branch remains or becomes relatively large, as compared with the stem vessel, the angle between the two approaches 0°, if relatively small, 90°. This is, in general, in agreement with the studies of W. Roux who has made elaborate mathematical estimations of the angle of branching, and finds that the relation is so precise that it can be expressed within limits, as a mathematical formula.

The growth in the length of vessels goes hand in hand with the increase in outside tissue, and is clearly, as Thoma has expressed in his second histomechanical law, brought about by the reaction of the vessel to the mechanical pull, exerted in a longitudinal direction on the vessel.

Thoma's third law, according to which the thickness of the

vessel-wall is regulated by blood-pressure, has not been tested in these studies.

In general, my findings are that, while blood-vascular endothelium differentiates, develops the power to grow by sprouting, and forms a primitive system of arteries, veins and capillaries as the result of hereditary factors, it very early becomes dependent, for its complete and orderly working out into the elaborate and beautifully proportioned adult vascular system, upon the regulative action of outside factors, to which it reacts in definite ways and upon which it comes to be completely dependent.

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THE FATE OF THE ULTIMOBRANCHIAL BODIES IN THE PIG (SUS SCROFA)[1]

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FOUR PLATES

I. INTRODUCTION

The fate of the ultimobranchial bodies is one of the many unsettled questions associated with the development of the thyroid gland. While most of the results of the considerable amount of investigation that has been done in recent years on these structures in various mammalian species have led to the interpretation that they do not contribute to the structural elements of the thyroid gland, there is still a diversity of views as to their actual fate. The variety of views expressed in the literature in regard to their fate in mammals is apparently due to several factors among which may be mentioned: (1) the possibility of a variable developmental behavior of these structures in different mammalian types, (2) inadequate series of successively older embryonic stages (especially embryos of larger mammals), and (3) faulty technique (principally poor fixation of the thyroid gland in the older embryos of the larger mammals, especially man).

[1] Some of the younger stages used in this study were prepared during the summer of 1914 while a guest in the Department of Histology and Embryology in Cornell University. I wish to express my appreciation to Prof. B. F. Kingsbury for the facilities so generously extended to me during that time.

A study of the ultimobranchial bodies in a wide range of successively older developmental stages of pig embryos of which the thyroid gland was well fixed, has resulted in bringing to light some interesting and important developmental features of these structures in this mammalian type.[2]

II. HISTORICAL

A brief historical outline representing in a general way the different views in regard to the fate of the ultimobranchial bodies is here given. The works of Verdun ('98) and Grosser ('12) render an extensive bibliography in this article unnecessary.

Born ('83) claims for the thyroid in the pig a triple origin. In a 21 mm. embryo he finds that the nuclei and cytoplasm of the cells composing the lateral thyroids stain more intensely than the cells of the median thyroid, but in a 37 mm. embryo in which the lateral thyroids have become imbedded in and fused with the median thyroid there is no histological difference between these structures and the median thyroid.

According to Moody ('12) in pig embryos 100 mm. in length no difference is to be observed "between the central and lateral parts of the gland in vascularity, colloid formation or connective tissue development." He believes that the ultimobranchial bodies contribute to the structural elements of the thyroid gland.

Simon ('96) claims that in mammals (guineapig, rabbit, cat, calf, sheep, and pig) the lateral thyroids do not actually fuse with the elements of the median thyroid, although they become entirely inbedded in the latter. The lateral thyroids in early developmental stages show signs of growth and further development. During this period, which he designates the période d'activité, the lateral thyroids are broken up into cell cords and cell

[2] No consideration was given to the origin of the structure variously termed 'ultimobranchial body,' 'postbranchial body,' 'suprabranchial body,' 'telobranchial body,' and 'lateral thyroids.' The morphological value of these terms have been discussed by Greil ('05), Rabl ('09), Kingsbury ('14), and others. Throughout the descriptive part of this work the term 'ultimobranchial body' will be used.

masses. This is brought about in an entirely passive way, by the ingrowth of vascular tissue and of elements from the median thyroid. These cell cords and cell masses stain more intensely than do the elements of the median thyroid. Traces of the lumen persist mainly in the more central part of the lateral thyroids. These structures in later developmental stages undergo degenerative changes. This period, which he designates the période de survivance, is characterized by a disappearance of the cell cords and the degeneration of the more centrally located epithelial cells, forming cysts lined with cuboidal or columnar epithelium which may or may not be ciliated. He also claims that the formation of cysts in the lateral thyroids of pig embryos is not a constant occurrence. Cysts in these structures in the pig were found only in five out of eleven embryos which he examined. In a 210 mm. embryo, the largest examined, no traces of the lateral thyroids were found. He is of the opinion that these structures in the pig disappear entirely.

Rabl ('08) finds that in the older mole embryos the lateral thyroids are reduced to insignificant structures, being represented by cell cords and cysts.

Verdun ('98) finds that in birds (chicken and duck) the postbranchial bodies remain independent structures of a glandular character but do not produce colloid. He regards these structures as special glands for birds. In the thyroid of mammals (rabbit, cat, dog, mole) the postbranchial bodies are represented by cysts and cell cords. The cysts vary greatly in size in the different species studied. Neither during the embryonic nor the postnatal life of these mammals was he able to demonstrate the transformation of the epithelial cords of the postbranchial bodies into thyroid follicles. He believes that the cysts and cell cords represent atrophied vestiges of the special gland in birds.

According to Tourneaux and Verdun ('97) the lateral thyroids in human embryos can for some time be recognized as a rather densely staining mass on the posterior surface of the lateral lobes of the thyroid gland. They undergo the same structural changes as the median thyroid, but more slowly. From the

median thyroid anlage, however, is derived the larger part of the structural elements of the thyroid gland.

According to Christiani, in rodents (rat) the lateral thyroid gives rise to an epithelial body.[3]

Maurer ('99) finds that in the Echidna the postbranchial bodies do not fuse with the median thyroid anlage. In the adult condition the thyroid lies posterior to the postbranchial bodies. The latter are represented by two alveolar structures which developed colloid (judged by staining reaction). The first traces of colloid formed in the postbranchial bodies appears, however, in later developmental stages than does that of the median thyroid.

Prenant ('94) finds that in sheep embryos the lateral thyroid develops into a central canal with an irregular lumen from the walls of which cell cords and cell masses (recognized by their dense structure) extend into the substance of the median thyroid. An intimate fusion takes place between the lateral and medial elements. In later developmental stages the tissue, which in earlier stages can be recognized as derived from the lateral thyroids, disappears. He was unable to determine whether or not the lateral thyroids contribute to the structural elements of the gland.

In this brief historical sketch the following views as to the fate of the ultimobranchian bodies were brought out: (1) They contribute to the structural elements of the thyroid gland; (2) They develop into cysts; (3) They develop into a gland of a different structure from that of the thyroid gland; (4) They develop into epithelial bodies, and (5) They disappear entirely.

III. MATERIAL AND METHODS

The material used for this investigation was collected in great abundance at a packing house. The upper jaw, cranium, and thorax were removed from embryos ranging from 15 to 25 mm. in length. The part containing the thyroid was thus made comparatively small and fixed well. From embryos 26 to 75 mm. in

[3] Cited from Zuckerlandl ('03).

length only the neck, from which the sides and the cervical vertebrae were removed, was reserved. From embryos 100 to 270 mm. in length (full term) only the thyroid with some of the surrounding structures—trachea, esophagus, a portion of the thymus, etc., was removed. The length in millimeters of the different developmental stages of which the thyroid was prepared for a study of the ultimobranchial bodies is as follows: 15, 16, 17, 17, 18, 18, 19, 19.5, 20, 20, 21, 21, 21.5, 22, 22, 23, 23, 23, 24, 24.5, 25, 25, 26, 27, 27, 28, 29.5, 30, 33, 35, 37.5, 38, 40, 48, 53, 60, 65, 65, 75, 100, 100, 111, 125, 125, 145, 150, 160, 175, 225, 245, 270, and 270. These figures represent the length of the embryos while in a fresh condition.

The fixing fluids employed were Zenker's fluid, Zenker-formol, and Picro-aceto-formol. The material was imbedded in paraffin. The earlier embryos were cut transversely in sections 5 microns thick, while those of later stages in sections 8 to 10 microns thick. Various stains were used. For embryos 15 to 65 mm. in length, iron hematoxylin gave the best results. The thyroid gland of later developmental stages was stained with Chloral hematoxylin and eosin, and eosin-methylene blue.

IV. DESCRIPTION OF STAGES

The earliest stage chosen for description is one just before the ultimobranchial bodies have fused with the thyroid gland. These structures will be described in two embryos of the same size only when there is a marked contrast in their size, structure, or position in the thyroid in the two embryos.

Embryo of 18 mm. (fig. 1). The ultimobranchial bodies have lost their connection with the fourth (?) pharyngeal pouch and extend cephalad beyond the anteror margin of the thyroid gland. Their anterior portion is in form a slender tube, oval in cross section, and with wall two to three layers of cells (nuclei) thick. Caudalward the walls of these structures gradually becomes thicker. In the portion in relation to the thyroid gland the lumen in places is obliterated and the remnants persist as mere slits. Anteriorly the surface of these tubules is quite smooth, while caudally irregularities occur on their surface. Their cau-

dal halves lie at varying distances dorsal to the lateral margin of the thyroid gland which has the general shape of a crescent with its horns directed anteriorly and dorsally. In a few sections they are separated from the thyroid only by a very thin layer of connective tissue (*U*). They extend almost to the caudal margin of the thyroid gland. Their caudal ends lie more closely together than their anterior ends.

The ultimobranchial bodies at this stage are composed of a syncytium. No cell walls are present. Vacuoles are found throughout their entire extent, although their distribution is not uniform. In places they can be found throughout an entire cross section of an ultimobranchial body while in other places they are confined to its more central portion. The vacuoles vary in size, the largest being almost as large as some of the nuclei.

The nuclei vary somewhat in size and in shape. Some are oval, some round, while others are irregular in outline. They contain from one to three nucleoli and a rather generous amount of chromatin which is in the form of granules and threads. The more centrally located nuclei have no regular arrangement while those near the periphery are in places quite regularly arranged. They are more closely packed together in the nonvacuolar than in the vacuolar portions of the ultimobranchial bodies. A consideration of this feature is of particular importance in stages in which the ultimobranchial bodies have fused with the thyroid gland. Mitoses of the nuclei can readily be found, especially in the larger more caudal part of the bodies, thus indicating a growth tendency of these structures. Neither blood vessels nor connective tissue are present in the ultimobranchial bodies at this stage.

The thyroid (*T*) is composed of nonvacuolar cell masses and cell cords[4] the latter of which are for the most part transversely arranged. No cell walls can be demonstrated, hence the cell

[4] Norris ('16) finds that in early developmental stages of human embryos the cell cords seen in cross sections of the thyroid gland represent in reality sections of fenestrated epithelial plates. As I have not made a careful study of the formation of the follicles in the thyroid gland I shall use the term 'cell cords' which is the microscopic picture presented in cross sections of the gland.

cords and cell masses have a syncytial structure. The nuclei vary in shape and somewhat in size but their form, average size, and structure in this stage is the same as in the ultimobranchial bodies. The nuclei of the thyroid are more closely packed together than in the vacuolar portions of the ultimobranchial bodies, but when a nonvacuolar portion of the latter is brought into the same microscopic field with a portion of the thyroid gland, no difference in structure can be seen between them even under high magnification (1500 diameters). Some of the spaces between the cords of cells are lined with endothelium and contain blood.

Embryo of 19.5 mm. (figs. 2 a and 2 b). The ultimobranchial bodies extend slightly farther cephalad than the thyroid gland. Only slight traces of their lumen still persist. They lie along the dorsal surface of the thyroid gland but are located nearer the mesial plane than those of the preceding stage. In some places there is actual fusion between these structures and the thyroid gland (fig. 2 a, right side), while in other places a thin layer of connective tissue intervenes (fig. 2 a, left side). The ultimobranchial body on the left side extends almost to the caudal margin of the thyroid gland (fig. 2 b), while on the right side it terminates twelve sections (5 microns in thickness) earlier. The shape and orientation of the thyroid gland is similar to that in the preceding stage.

In this stage, as in the preceding one, both vacuolar and nonvacuolar areas are found in places along the periphery of the ultimobranchial bodies. In some places where actual fusion has taken place with the thyroid gland it is impossible to tell where the two structures meet. Fusion with the thyroid gland has taken place along the ventro-lateral surface of the ultimobranchial bodies. The dorso-medial surface of these structures is in places studded with epithelial buds (fig. 2 a).

In the ultimobranchial bodies of this developmental stage are found a few nuclei in which the nucleoplasm stains quite deeply in comparison with that in the large majority of nuclei present. In some of these nuclei the chromatin is more abundant than in the more numerous and more lightly stained ones but in both

types of cells it is distributed in the form of threads and granules. With comparatively low magnification they appear as dark specks among the other nuclei (fig. 2 a). Since these have apparently been regarded by some investigators as degenerating nuclei, they deserve special attention in successively older developmental stages.

Embryo of 20 mm. (figs. 3 a, 3 b, and 3 c). The ultimobranchial bodies are small anteriorly and extend slightly farther cephalad than the thyroid gland. The one on the left side is separated for a short distance from the extreme anterior part of the thyroid (fig. 3 a). Caudalward these structures rapidly become larger and form the greater portion of the horns of the crescent shaped tripartite complex. The one on the left side is slightly larger, and eleven sections (5 microns in thickness) longer than the one on the right side and extends as far caudally as does the thyroid gland. The extreme caudal portion of these structures is not fused with the thyroid gland. Remnants of the lumen are present in two places in the anterior third of the left one.

A feature quite noticeable in the ultimobranchial bodies of this developmental stage is the presence of unusually small nuclei which are found in small groups and promiscuously scattered among those of usual size. From their similarity in structure to the larger nuclei they seem to be normal. Deeply stained nuclei, which are somewhat more numerous throughout these structures than in the preceding stage, are also present in these groups.

It is impossible to determine definitely the exact place of fusion between the ultimobranchial bodies and the thyroid gland. Judging, however, from the uniformity of the distribution of the deeply stained nuclei, from the absence of cell cords along the greater portion of their dorso-mesial free border, from the manner in which they terminate, as stated above, from the absence of blood vessels, and from the distribution of the small nuclei and vacuoles, it seems that the cell masses labeled ultimobranchial bodies in the figures 3 a, 3 b, and 3 c represent exclusively the ultimobranchial bodies.

Epithelial buds, as represented in figure 3 b (*Ep.B.*), are present in various places along the free border of these structures. These buds are fused to the more or less vacuolar mass of cells. Mitosis can be found without much searching in both the ultimobranchial bodies and the thyroid gland.

Embryo of 21 mm. (fig. 4). Both ultimobranchial bodies are as long as the thyroid gland. They are fused to the latter along their entire extent excepting the extreme caudal end of the left one which is separated from the gland by a thin layer of connective tissue. The one on the right side has a comparatively regular outline and makes up nearly all of the lateral portion of the tripartite complex (*U*). The ultimobranchial body on the left side is more deeply embedded in the thyroid gland than the right one which makes it difficult to follow its extent in transverse sections. In places blunt and both vacuolar and nonvacuolar epithelial buds are attached to these structures.

Groups of small nuclei in the ultimobranchial bodies are present but they are not as mumerous as in the preceding stage. The darkly stained nuclei are no more numerous than in the previous stage. A few darkly stained nuclei were found in the cell cords of the thyroid gland. These have a structure similar to the darkly stained nuclei of the ultimobranchial bodies but are not nearly as numerous and can be found only after prolonged searching. Mitoses can readily be found in all the different components of the tripartite complex.

Embryo of 21.5 mm. (fig. 5). The ultimobranchial bodies lie along the entire extent of the dorso-medial margin of the thyroid gland and compose the largest portion of the tripartite complex. The anterior extremity of both ultimobranchial bodies and the posterior extremity of the left one are not fused with the thyroid gland. Their largest diameter (*U*) is about midway between their extremities from which they gradually taper to blunt points. Their greater portion is vacuolar but nonvacuolar areas are present in their deeper parts as well as along their periphery. Large blunt epithelial buds, some of which are vacuolar, are present in various places along their free border. The darkly

stained nuclei and groups of small nuclei are more numerous than in the preceding stage.

Embryo of 22 mm. (figs. 6 a and 6 b). The tripartite complex presents extremely varied pictures. Its anterior fourth is composed entirely of typical thyroid cell-cords while its caudal portion is composed chiefly of the ultimobranchial bodies (fig. 6 b). The caudal portion of each ultimobranchial body is composed of a cell mass of irregular outline in which remnants of the lumen, lined with columnar epithelium, still persist (fig. 6 b, *L*). Anteriorly, they are largely broken up into coarse cell cords which process marks the beginning of important developmental features in these structures (fig. 6 a, *U*). Nonvacuolar areas can be found throughout their entire extent. The deeply stained nuclei in the coarse cell cords, in the more central unbroken masses, and in the epithelial buds are more numerous than in any of the preceding stages. They can be quite readily found in the cell cords of the thyroid gland (figs. 6 a and 6 b), but are not nearly as numerous as in the ultimobranchial bodies. No degenerating nuclei, such as pyknotic or fragmented nuclei, were found. Small nuclei, in groups and diffusely scattered, are more numerous in the ultimobranchial bodies of this stage than in the previous one. In another 22 mm. embryo the size, shape, and extent of the ultimobranchial body along the thyroid gland is quite similar to that of the 21.5 mm. embryo described above.

Embryo of 23 mm. (figs. 8 a, 8 b, and 8 c). The ultimobranchial bodies are small anteriorly and extend slightly farther cephalad than the thyroid gland. For a short distance anteriorly the ultimobranchial bodies and the thyroid are not fused. From their point of fusion with the thyroid they rapidly become larger so that the caudal portion of the tripartite complex is largely composed of the ultimobranchial bodies (figs. 8 a, 8 b, and 8 c, *U*). The epithelial buds attached to the ultimobranchial bodies are in general not as large as those in the 22 mm. embryo. The darkly stained nuclei and groups of small nuclei are also less numerous than in the preceding stage. Only an occasional darkly stained nucleus can be found in the cell cords of the thy-

roid gland. A few blood vessels of a capillary character are found in the larger portion of the ultimobranchial bodies.

Embryos of 24 to 30 mm. During this developmental period quite marked changes occur in the ultimobranchial bodies, the most pronounced of which is a breaking up of their major portion into cell cords which, when first formed, are usually larger than those of the thyroid gland. Two factors are apparent during the formation of cell cords, namely, a continued growth and division of the epithelial buds, and their invasion by mesenchymal and vascuolar connective tissue. The extent to which this process occurs during this developmental period varies. In some embryos these structures are almost entirely broken up into cell cords while in others a centrally located, more or less vacuolar and irregularly outlined core, variable in size, persists for some time longer. This process is illustrated in figure 9 (*U*), which represents a section through almost the middle portion of the tripartite complex in a 27 mm. embryo. In most stages of this developmental period (24 to 30 mm.), and even in some later stages, the caudal portion of the ultimobranchial bodies is for a time less broken up into cell cords than their more anterior part. Also, these structures never become entirely vacuolar. Some of the coarse cell cords are composed of a nonvacuolar syncytium. Nonvacular areas are also present in the more centrally located syncytial mass and in the larger and less unbroken caudal portion of these structures. Groups of small nuclei which appear normal in structure are present in both vacuolar and non vacuolar parts. In places, instead of being arranged in groups, the small nuclei are quite uniformly scattered among the larger nuclei. Mitoses in both the thyroid gland and ultimobranchial bodies can readily be found.

It is during this developmental period and also in somewhat earlier and later stages that the darkly stained nuclei are most numerous. In only two developmental stages, namely, in a 23 mm. embryo (not the one described above), and in a 24 mm. embryo (fig. 7, *D.N.*), were degenerated (pyknotic and fragmented) nuclei found in sufficient numbers to suggest a general degeneration of these structures. The degenerated nuclei in

these stages were not generally distributed throughout the ultimobranchial bodies but were found in localized areas. A few darkly stained nuclei can be found in the cell cords of the thyroid gland during this developmental period. In the thyroid, however, they are never very numerous and in some they are found only after prolonged searching.

Embryo of 29.5 mm. (figs. 10 a, 10 b, and 10 c). The tripartite complex in this embryo is of interest in that a large portion of it is asymmetrical in shape, due to the unequal length of the ultimobranchial bodies. Nearly all of the anterior fourth of the complex is symmetrical and is composed of cell cords of the thyroid gland only (fig. 10 a). The greater portion of the middle two-fourths of the tripartite complex is characterized by the presence of massive cell cords of the left ultimobranchial body and the entire absence of the right ultimobranchial body (fig. 10 b). Along the posterior fourth of the thyroid gland both ultimobranchial bodies are present. The left one terminates rather abruptly thirty sections (150 microns) anterior to the caudal end of the thyroid while the right one tapers to a point and extends as far caudally as the thyroid gland. The extreme caudal portion of each ultimobranchial body is less broken up into cell cords than is represented in figure 10 c. Small disconnected vacuolar areas are present in the more caudal portion of both branchial bodies. The large cell cords are almost entirely free from vacuoles but are characterized by a comparatively large number of small nuclei. The deeply stained nuclei, which are comparatively few in number, are most confined to the ultimobranchial bodies. Only a few are present in the cell cords of the thyroid gland. Only a very few degenerated nuclei were found.

In embryos from about 30 mm. in length to stages in which colloid is first present in the follicles of the thyroid gland (75 mm.), the ultimobranchial bodies present a varied appearance. They are largely broken up into cell cords and in the progress of development the cell cords of the thyroid gland and usually those of the ultimobranchial bodies have become closely packed together so that a sharp demarcation between the

median and lateral elements of the tripartite complex is not always evident. A description of a few stages will suffice to bring out the general character of the ultimobranchial bodies during this developmental period. Since the thyroid gland in previous stages is free from vacuoles, it is, I believe, safe to assume that the vacuolar areas found in the succeeding stages represent portions of the ultimobranchial bodies.

Embryo of 33 mm. The ultimobranchial bodies are limited to the posterior half of the tripartite complex. They are located on each side of the median plane, deeply buried beneath the dorsal surface of the thyroid gland and are represented by disconnected vacuolar areas the majority of which are not sharply circumscribed but gradually give place to the compactly arranged cell cords of the thyroid gland with which they are fused. The thyroid terminates posteriorly in two short blunt processes. In these processes small vacuolar areas are promiscuously scattered among the cell cords. A few small vacuolar areas which are round in cross section and sharply demarcated by connective tissue from the surrounding cell cords were also found. Only a few darkly stained nuclei are present. No degenerated nuclei were found.

Embryo of 35 mm. The only traces of the ultimobranchial bodies are small disconnected vacuolar areas on each side of the median plane of the thyroid gland. The gland terminates posteriorly in two short blunt processes of nearly equal length, both of which are partly vacuolar. Only a few deeply stained nuclei are present.

Embryo of 37.5 mm. The anterior portion of the tripartite complex is very large and strongly crescent in outline. Caudalward it gradually loses its crescent outline and ends in a single blunt cone-shaped process. The ultimobranchial bodies lie in the posterior four-fifths of the thyroid gland. Their anterior ends lie imbedded beneath the dorsal surface of the thyroid lateral to its median plane. Caudalward they rapidly increase in size and shift in position so that in places they extend to the free surface on the lateral margin of the thyroid gland. Their posterior ends are fused and compose by far the largest part of the

caudal one-fifth of the tripartite complex. The greater portion of the ultimobranchial bodies are in the form of vacuolar syncytial cores which give off coarse cell cords. Some of the coarse cell cords are vacuolar and many are fused to the cell cords of the thyroid gland. The central core is more or less invaded with mesenchymal and vascular connective tissue. Deeply stained nuclei are quite numerous in the ultimobranchial bodies and a few are found in the cell cords of the thyroid gland. No degenerated nuclei were found. The variableness in the size of the nuclei in the ultimobranchial bodies is more marked than in the nuclei of the thyroid gland, the former having a proportionally larger number of small nuclei. The extent to which the transformation of the ultimobranchial bodies has taken place in this stage is about equal to that in the 29.5 mm. embryo.

Embryo of 38 mm. The ultimobranchial bodies are limited to the posterior two-thirds of the tripartite complex. Their anterior ends are small and entirely imbedded in the thyroid gland near its dorsal surface. Caudalward they rapidly increase in size. The tripartite complex ends in two blunt cone-shaped processes the greater portion of which are composed of the ultimobranchial bodies. The ultimobranchial bodies are composed of irregularly outlined syncytial cores which gradually merge into the compactly arranged cell cords of the thyroid gland. Only a few capillaries are found in them. Mitoses in the vacuolar areas as well as in the cell cords of the thyroid are quite numerous. Only a few deeply stained nuclei are present. No degenerated nuclei were found.

Embryo of 40 mm. The ultimobranchial bodies lie in the posterior half of the thyroid gland. Their anterior parts are represented by small disconnected vacuolar areas which lie deeply buried in the thyroid gland lateral to its median plane. Caudalward these areas become large and branched so that the caudal fifth of the thyroid gland is largely invaded by a vacuolar syncytial mass which is not sharply demarcated from the closely packed cell cords of the thyroid gland. The tripartite complex ends in two blunt and slightly vacuolar processes of unequal length. A small number of deeply stained nuclei are present

throughout the entire complex. Only a few degenerated nuclei were found in the vacuolar areas. There are realtively more small nuclei present in the vacuolar areas than in the nonvacuolar portions and mitoses are more numerous in the latter than in the former areas.

Embryo of 48 mm. (figs. 11 a and 11 b). Both ultimobranchial bodies extend anteriorly as far as the thyroid gland. The one on the right side is isolated from the anterior fourth while the one on the left side is isolated from the anterior third of the thyroid gland (fig. 11 a, *U*). Excepting a vacuolar area present in the anterior end of the left one and traces of the lumen caudal to the vacuolar area, the isolated portions of the ultimobranchial bodies have a structure identical to that of the thyroid gland along which they lie. The right ultimobranchial body near its fusion with the thyroid is quite large (fig. 11 a, *U*). The part fused to the thyroid gland is difficult to follow, yet traces of it may be seen in the form of small vacuolar areas that are promiscuously scattered beneath the dorso-lateral margin of the tripartite complex. Some of these areas are sharply outlined while others gradually merge into the compactly arranged cell cords.

The left ultimobranchial body caudal to its most anterior point of fusion with the thyroid gland is characterized in some places by very irregular vacuolar areas, while in other places by large, closely packed cell cords. In some places also it is only partially fused to the thyroid gland while in other places it is entirely separated from it by connective tissue (fig. 11 b, *U*.) Traces of the lumen still persist (*L*). The tripartite complex ends in two large conical processes which have, excepting a small vacuolar area found in each, a typically thyroid structure. Only a few darkly stained nuclei were found. Mitoses throughout the entire complex can be found without much searching.

Embryo of 53 mm. (fig. 12). Traces of the ultimobranchial bodies are present in the caudal half of the tripartite complex. The anterior end of each is represented by a small irregularly outlined vacuolar area which lies lateral to the median plane just below the dorsal surface of the gland. For some distance

caudalward these areas become larger, in places very irregular in outline, in places broken up with typical thyroid structures, and are located more deeply in the lateral halves of the thyroid gland. The thyroid ends in a single blunt process that has a typically thyroid structure. In a few places the ultimobranchial bodies are unusually vacuolar. In these places the nuclei do not stain deeply (*U*). Similar lightly stained areas were observed by Kingsbury ('14) in the thyroid gland of human embryos. Also, a few groups or nests of small, closely packed nuclei were found. In some of these groups the nuclei had a normal structure, while in others they were only slightly stained. A few degenerated nuclei were found in the vacuolar areas and in their immediate neighorhood. Deeply stained nuclei are present in small numbers in both vacuolar and nonvacuolar parts.

Embryo of 60 mm. (fig. 13). The ultimobranchial bodies are limited to the posterior third of the thyroid gland. The right one is fused to the dorso-lateral margin of the gland and along its greater extent is composed of loosely arranged cell cords (*U*). Near the caudal end of the thyroid it merges into a vacuolar area which is interspersed with typical thyroid structures. The nuclei of the cell cords are on an average smaller than those found in the thyroid gland but, excepting a few darkly stained nuclei, they have a normal structure. The left ultimobranchial body lies just lateral to the median plane and is more deeply imbedded in the thyroid than the right one. It is largely composed of loosely arranged cell cords. Small vacuolar areas are present throughout its entire extent. The tripartite complex ends in a single process which is partly vacuolar. Mitoses can readily be found in the loosely arranged cell cords.

Embryos of 65 mm. The ultimobranchial bodies in two embryos of this developmental stage are described in order to contrast the structure of these bodies in two embryos of the same age. In one embryo these structures are represented by small disconnected vacuolar areas which are promiscuously scattered in the caudal half of the tripartite complex. In the other embryo the ultimobranchial bodies are located in the posterior third of the thyroid gland, and each one is characterized by a large and

very irregularly outlined and continuous vacuolar mass to which coarse and loosely arranged cell cords, some of which are vacuolar, are attached. In the extreme caudal portion of the thyroid gland these structures fuse with each other and make up a large portion of its blunt termination. In each embryo a few deeply stained nuclei and a few degenerated nuclei were found.

Embryo of 75 mm. This is the youngest developmental stage in which colloid is found in the thyroid gland (picro-aceto-formol and hematoxylin and eosin). The follicles containing colloid are not numerous but are quite uniformly distributed throughout the anterior portion of the gland. The ultimobranchial bodies are limited to the posterior two-thirds of the gland lateral to the median plane and bordering the dorsal surface of the gland. They are represented by a continuous area of cell cords which contains no colloid. Within these areas are found small, irregularly outlined, and disconnected syncytial masses which contain an unusually large number of small nuclei. These nuclei have the same structure as those found in the cell cords of the thyroid gland. Many of the cell cords which do not contain colloid are fused to these syncytial masses. Vacuoles are almost entirely lacking. A few degenerated nuclei are present, found only after prolonged searching. The tripartite complex ends in two blunt processes which have a typical thyroid structure.

Embryos of 100 mm. The tripartite complex of two embryos deserves notice. In both the colloid is more abundant than in the previous stage.

Embryo No. 1 (fig. 14). The ultimobranchial bodies are limited to the middle two-fourths of the thyroid gland. The right one lies partially exposed on the dorsal surface of the thyroid lateral to its medial plane. In some places it is composed of coarse and loosely arranged cell cords (*U*), while in other parts the cell cords merge into a large syncytial mass. In places the connection between it and the thyroid is more intimate than is shown in figure 14. In the syncytial masses the nuclei are on an average a little smaller than those in the cell cords of the thyroid, but in both their structure is the same. No vacuoles are

present. A few deeply stained nuclei are present. Some are also found in the cell cords of the thyroid gland. A few degenerated nuclei were found. The ultimobranchial body on the left side has a similar structure to the one on the right side but lies deeply buried below the dorsal surface of the gland. The portion of the tripartite complex which can be distinctly recognized as a derivative of the ultimobranchial bodies, and the cell cords in their immediate neighborhood contain no colloid although the cell cords have a typical thyroid structure of somewhat earlier stages.

Embryo No. 2. The ultimobranchial bodies cannot be identified structurally. However, it is to be noted that on the right side, lateral to the median plane and along the dorsal border in the middle third of the gland is an area which contains no colloid. This area is composed of closely packed cell cords which have the same structure as the cell cords of the thyroid just before the appearance of colloid, such as in a 60 mm. embryo. On the left is an area similar in structure to the one on the right side only its cephalo-caudal extent is considerably less. These areas which are free from colloid correspond favorably in position to that of the ultimobranchial bodies in some of the previous stages. The thyroid terminates in two rather blunt processes the extreme caudal portions of which contain no colloid.

Embryo of 111 mm. The ultimobranchial body on the right side of the gland is represented by two small and widely separated syncytial masses which extend through a series of ten and six sections respectively (10 microns in thickness), and on the left side it is represented by a syncytial mass extending through a series of eleven sections. These syncytial masses are quite vacuolar and the nuclei are comparatively small and clear and many are irregular in outline. Only a few darkly stained and degenerated nuclei are present.

It is of importance to note that on the right side lateral to the median plane in the middle two-fourths of the gland and in line with the syncytial masses described above is an irregularly outlined area of closely packed cell cords. This area is quite large in cross section and is free from colloid. A similar area of about the same length is present in the left side of the thyroid but it

extends farther caudally and not so far anteriorly as the right one. These areas which are free from colloid correspond favorably in position to that of the ultimobranchial bodies in some earlier developmental stages.

Embryos of 125 mm. The ultimobranchial bodies in two embryos of this developmental stage were examined.

Embryo No. 1 (fig. 15). The ultimobranchial body on the right side lies in the middle two-fourths of the thyroid gland. Its anterior end is deeply imbedded beneath the dorsal surface of the thyroid and is composed of a very irregularly outlined and vacuolar syncytial mass in which the nuclei have about the same size as those in the follicular epithelium of the thyroid. A few pale nuclei are present. Cell cords, some of which are coarse and vacuolar, lead from the vacuolar area and are fused with the surrounding thyroid structures. Slightly farther caudal it reaches the free surface on the ventro-lateral side of the gland and is composed of a loose network of cell cords some of which are vacuolar. From this place it gradually occupies a more dorsal position in the thyroid gland and is composed of closely packed cell cords, having a structure similar to that of the thyroid gland just before the appearance of colloid. Its more caudal portion reaches the free surface of the thyroid gland on its dorsal aspect (*U*) and contains a large cyst (*C*).

The ultimobranchial body on the left side is represented by a series of six small disconnected, and irregularly outlined syncytial masses which lie just lateral to the mesial plane of the gland. These areas are more or less vacuolar and do not contain any colloid. The thyroid ends in a single process throughout which the colloid is quite uniformly distributed.

Embryo No. 2 (figs. 16 a and 16 b). The tripartite complex of this embryo is of interest in that the ultimobranchial bodies are only partially imbedded in the thyroid gland. The ultimobranchial body on the right side lies along the lateral margin of about the middle two-fourths of the thyroid gland to which it is fused. It is fusiform in shape, with its greatest diameter about midway between its ends (fig. 16 a, *U*). The free portion along its entire extent is composed of syncytial masses and coarse and tortuous cell cords in both of which are found cystoid follicles which are

lined with cuboidal and columnar epithelium. Some of the nuclei in the syncytial masses and in the coarse cell cords stain more deeply than others, and in general they are more variable in size than those in the follicular epithelium of the thyroid gland. The nuclei in the epithelial lining of the cystoid folicles lie closely together and stain uniformly. While the cystoid follicles are free from colloid in this developmental stage, small follicles containing colloid are thinly scattered throughout its free portion (fig. 16 a, *Co*). Along the line of fusion of the free portion of the ultimobranchial body to the thyroid gland there is in the latter an area composed of cell cords in which colloid is just beginning to form (fig. 16 a). The cell cords of this area have a structure similar to those in earlier stages in which colloid formation has just begun. In the free portion of this structure vacuoles are almost entirely lacking and only a few degenerated nuclei were found.

The length of the ultimobranchial body on the left side is equal to the length of the right one. It also lies along the lateral margin of the thyroid gland but is more deeply imbedded in it (fig. 16 b, *U*). In cross section it is smaller than the right one but, excepting the absence of cystoid follicles, it has a similar structure. By referring to the figure it will be seen that it merges gradually into the thyroid gland. The follicles containing colloid gradually become smaller toward the more central portion of the ultimobranchial body in which only an occasional small follicle can be found.

Embryo of 145 mm. (fig. 17). The ultimobranchial bodies on both sides are limited to the middle two-fourths of the thyroid gland just lateral to its median plane. The right one along nearly its entire extent is partly exposed to the free surface along the dorsal border of the gland. The portion most deeply imbedded in the thyroid gland is represented by an area of cell cords in which the follicles containing colloid are quite numerous but all very small (*U*). In places along its free margin are found cystoid follicles which also contain colloid (*C.F.*).[5]

[5] The substance in the cystoid follicles is called 'colloid' on the ground that it has a staining reaction identical to that of the colloid in the follicles of the thyroid gland.

The ultimobranchial body on the left side is exposed to the free surface only in a few places. The larger portion lies immediately beneath the dorsal border of the thyroid gland. In one of its exposed parts are found cystoid follicles which contain colloid. The imbedded parts have the same structure as the imbedded portion of the right one. No darkly stained nuclei were found in either of the ultimobranchial bodies.

Embryo of 150 mm. The ultimobranchial bodies are located in the middle and a part of the posterior third of the thyroid gland near its lateral borders. They are represented by areas of typical thyroid structures in which the follicles containing colloid are small and not very numerous. In places they reach the free surface of the gland along its lateral border. No cystoid follicles or deeply stained and degenerated nuclei are present.

Embryo of 160 mm. (fig. 18). The only structures present in the thyroid gland indicative of the presence of the ultimobranchial bodies are areas (*U*), on each side lateral to the median plane along the dorsal surface of the gland. In these areas the follicles containing colloid are quite small in comparison to the large majority present in the thyroid gland, but appreciably larger than those found in corresponding areas in the thyroid of 145 and 150 mm. embryos. These areas extend from about the caudal end of the anterior third well into the posterior fourth of the thyroid gland which terminates in a rather blunt single process. In the caudal end are a very few large follicles containing colloid but it was impossible to determine whether or not they developed in connection with the ultimob ranchial bodies. No darkly stained or degenerated nuclei were found.

Embryo of 175 mm. The follicles in the thyroid gland are on an average considerably larger than those found in the preceding stage. They vary greatly in size but are uniformly distributed throughout the gland. No structures are present which can be interpreted as derivatives of the ultimobranchial bodies.

Embryo of 225 mm. (fig. 19). The only apparent traces of the ultimobranchial bodies are areas of considerable extent in which the follicles are comparatively small (*U*). These areas are located in the middle third on each side of the median plane and

along the dorsal surface of the thyroid gland, and compare in position to the ultimobranchial bodies in some other comparatively late developmental stages. The areas of small follicles on the right side is a little shorter than that on the left side.

Embryo of 245 mm. (fig. 20). On the right side along the lateral margin of the posterior two-thirds of the thyroid gland is an area containing many cystoid follicles which contain colloid and which are lined with cuboidal epithelium. This area, small anteriorly, gradually becomes larger and reaches its greatest cross-section area near the posterior fourth of the thyroid gland. From this position it decreases in size and near its termination it is almost separated from the thyroid gland. This area occupies a position similar to that of the ultimobranchial bodies in some earlier stages and apparently represents a partially imbedded ultimobranchial body similar to the right one in No. 2 of the 125 mm. embryo (fig. 16 a).

On the left side lateral to the median plane and below the dorsal surface of the thyroid gland is an area in which the average size of the follicles is appreciably smaller than the large majority of follicles in other portions of the thyroid gland. This area lies in the posterior half of the thyroid gland but does not extend as far caudally as the area of large follicles on the right side. It also corresponds favorably in position to that most generally occupied by the ultimobranchial bodies in earlier stages.

Embryos of 270 mm. (full term). The thyroid glands of two full term embryos were examined.

Embryo No. 1. The follicles containing colloid are variable in size but uniformly distributed throughout the gland. The only portion of the gland which can be interpreted as a derivative of an ultimobranchial body is an area of only small follicles on the right side lateral to the median line in the posterior half of the gland. This area extends through a series of only sixty sections (10 microns in thickness) and lies hear the dorsal surface of the gland.

Embryo No. 2 (fig. 21). The thyroid gland extends through a series of 827 sections (10 microns in thickness). The left ulti-

mobranchial body is not completely transformed into typical thyroid structures. It lies in the posterior half of the gland and can be traced through a series of 234 sections (2.3 mm.). It is characterized by a small area of tortuous and nonvacuolar syncytial cords free from colloid which is eccentrically located in an area of small follicles (*U*). The nuclei in the syncytial cords correspond in size and structure to those in the follicular epithelium. A few nuclei in mitotic division are present. No deeply stained or degenerated nuclei are present.

The right ultimobranchial body extends through a series of 243 sections and is found in the middle third of the thyroid gland. It is characterized by an area of small follicles. In both ultimobranchial bodies from their more central portion toward their periphery the follicles gradually become larger. There is no sharp line of demarcation between these structures and the thyroid gland.

V. SUMMARY AND DISCUSSION

By comparing the rate of growth of the ultimobranchial bodies and the thyroid gland, it is seen that a more uniform size ratio is maintained in early than in later developmental stages. During this 'période d'activité' (Simon) of the ultimobranchial bodies, which extends from an 18 mm. or earlier developmental stage to about a 33 mm. stage, the cephalo-caudal extent of the ultimobranchial bodies is nearly or entirely equal to that of the thyroid gland. In later stages (33 mm. to full term) in which the ultimobranchial bodies can be recognized structurally, their cephalo-caudal extent is generally much less than that of the thyroid gland, which indicates that in later developmental stages the rate of growth of the thyroid exceeds that of the ultimobranchial bodies. In embryos from about 50 mm. in length to full term the ultimobranchial bodies are usually located in the posterior half of the thyroid gland. In a few stages they occur in the middle third or the middle two-fourths of the gland. Simon ('96) claims that during this period of retarded growth of the ultimobranchial bodies, which he calls the 'periodé de survivance,' they undergo degenerative changes which is manifested prin-

cipally by cystic formations (guinea-pig, rabbit, cat, calf, sheep) or their complete disappearance (pig).

The ultimobranchial bodies first fuse with the thyroid gland along their ventro-lateral border. In early stages (19 mm. to about 27 mm.) they make up a considerable portion of the horns of the crescent-shaped tripartite complex. The extent of their fusion to the thyroid gland during their period of retarded growth (from about 33 mm. to full term) is variable. In the majority of late stages they are entirely imbedded in the thyroid gland while in some they are only partially imbedded. The latter condition is particularly the case in the following embryos; 48 mm. (figs. 11 a and 11 b); 60 mm. (fig. 13); 100 mm. (fig. 14); 125 mm. (fig. 16 a and 16 b); and 145 mm. (fig. 17). In the later stages they usually lie more or less deeply imbedded below the dorsal surface of the thyroid gland lateral to its medial plane, but occur less frequently along the lateral or dorso-lateral margin of the gland.

The formation of vacuoles in the ultimobranchial bodies begins before their fusion with the thyroid gland has occurred and continues after fusion. However, in the various stages examined no ultimobranchial body was found that is vacuolar throughout. In human embryos Kingsbury ('14) finds that vacuolation, 'reticulation,' continues until the entire structure is altered in this way. The extent to which vacuolation takes place varies in embryos of the same and different developmental stages. For example in No. 1 of the 125 mm. embryos the more central portion of these structures are quite vacuolar while in No. 2 of the 125 mm. embryos no vacuoles are present. Also no vacuoles are present in the left ultimobranchial body in No. 2 of the 270 mm. embryos. In early stages non-vacuolar portions are present along the periphery as well as in the deeper portions of these structures. In later stages in which the ultimobranchial bodies are largely broken up into cell cords the vacuoles are most numerous in their more central unbroken portion although vacuolated syncytial cords were found. In a few stages of which the embryo 53 mm. long is an example, the only part of the ultimobranchial body that can be recognized structurally as such are

small vacuolar syncytial masses entirely surrounded by typical thyroid structures (fig. 12).

Up to about a 24 mm. stage a marked contrast exists in the structure of the ultimobranchial body and the thyroid gland, in that the former are largely unbroken syncytial masses, while the latter is broken up into cell cords (as seen in cross section). Although epithelial buds produce irregularities on the surface of the ultimobranchial bodies even in a 21 mm. stage and indications of cell cord formation were found in one 22 mm. embryo, the process of extensive cell-cord formation in these structures is particularly active in stages ranging from 24 to 27 mm. in length. The larger caudal end becomes broken up somewhat later than the smaller anterior end. Usually, also, the more central portion breaks up into cell cords later than the periphery. The syncytial cords when first formed are usually larger or coarser than those of the thyroid gland. Many are vacuolar for some distance away from the central more or less vacuolar core to which they may be attached. The time of breaking up of the central core into cell cords is very variable. The extent to which the ultimobranchial bodies become invaded with vascular tissue corresponds closely to the extent of cell cord formation. The first blood vessels, which are of a capillary nature, are found in these structures in a 23 mm. embryo.

According to Simon ('96) the cell cores of the ultimobranchial bodies are formed in an entirely passive way, namely, by the ingrowth of vascular tissue and of structural elements of the median thyroid. That the former is a potent factor in this process is, I believe, beyond doubt. It appears to me, however, that he lays too much stress on the formation of cell cords by the ingrowth of thyroid structures which will be considered later. Another active factor in the process of cell cord formation is a continued growth and branching of the epithelial buds found on their surface in early stages. The buds by continued growth and branching take the form of coarse cell-cords which can in many instances be recognized structurally from the smaller cell cords of the thyroid gland by the larger proportion of small nuclei which they contain and by vacuoles which, when present, are

found in their more proximal ends near their attachment to the more central unbroken portion of these bodies. Also, in stages in which the darkly stained nuclei are numerous many can usually be found in the coarse cell cords. The presence of nuclei in mitotic division in these cords is further evidence that they really grow.

The cell cords of the ultimobranchial bodies when first formed are generally more loosely arranged than those of the thyroid gland (figs. 9, 10 c, and 13). The time at which they become more compactly arranged and resemble in appearance the thyroid gland previous to the appearance of colloid in the latter, varies greatly. For example, in embryos of 48 and 53 mm. in length, excepting the small vacuolar portions, they have a structure, similar to the thyroid gland, while in both 125 mm. embryos cell cords in portions of these structures have still a quite loose arrangement.

The deeply stained nuclei are most numerous in the ultimobranchial bodies in stages from 20 mm. to about 30 mm. in length. In the first half of this brief developmental period (20 to 30 mm.) the ultimobranchial bodies attain their largest size as unbroken or solid structures while in the latter half of this period the process of cell cord formation is very active. The deeply stained nuclei diminish in number in stages beyond 30 mm. in length and finally disappear altogether. Their decrease in number is, however, not uniform in successively older stages. For example, in a 35 mm. embryo in which the only structural traces left of the ultimobranchial bodies are small disconnected vacuolar areas, the darkly stained nuclei are comparatively few in number, while in a 37.5 mm. embryo in which these structures are still large and easily traceable, the darkly stained nuclei are quite numerous. In late developmental stages in which the ultimobranchial bodes can be structurally recognized as such the darkly stained nuclei have largely or entirely disappeared. For example in No. 2 of the 125 mm. embryos there are some present although not in large numbers, while in the ultimobranchial bodies in No. 2 of the full term embryos no darkly stained nuclei are present.

The deeply stained nuclei have been regarded by Simon ('96) as degenerating nuclei. In only two developmental stages (23 and 24 mm. embryos, fig. 7) were degenerated nuclei found in sufficient number to suggest a general degeneration of these structures. In some of the later developmental stages degenerated nuclei were also found but always in small numbers. It appears that the degenerated nuclei are derived from the darkly stained nuclei although I was unable to trace their source through intermediate forms directly to them. Some of the nuclei in connective tissue cells, in developing muscle fibers, in epithelial cells lining the esophagus, and also in some stages in the cell cords of the thyroid gland, stain deeply. This gives them a structural appearance quite similar to those found in the ultimobranchial bodies. The presence of these nuclei in various developmental structures suggested the probability that the dark nuclei in the ultimobranchial bodies are in a certain physiological state. This, however, is mere conjecture. If they represented a general degeneration of the ultimobranchial bodies one would naturally expect to find large numbers of degenerated nuclei in later developmental stages, but a contrary condition is the case. They gradually decrease in number while the ultimobranchial bodies continue in their growth. This fact seems to me to be strong evidence in favor of the persistence of these structures.

A feature quite noticeable in the ultimobranchial bodies in most of the earlier developmental stages and in some of the quite late stages is the small and variable size of some of the ultimobranchial nuclei. Grosser ('10) and Kingsbury ('14) also observed small ultimobranchial nuclei in human embryos. The small nuclei are very variable in number in stages of about the same age. Some of these nuclei also stain deeply in stages in which deeply stained nuclei are present, and in a few instances groups of pale small nuclei were found. However, the large majority of the small ultimobranchial nuclei have a normal structure, in all stages in which they occur. In late stages no small nuclei are present. Although the significance of the darkly stained and the small ultimobranchial nuclei are unknown to me I am

convinced that they do not represent a general degeneration of the ultimobranchial bodies.

Follicles containing colloid appear first in the thyroid gland in a 75 mm. embryo. In the ultimobranchial bodies the follicles containing colloid are first quite numerous, though small (excepting the cystoid follicles), in a 145 mm. embryo (fig. 19). A few small follicles containing colloid were found in these structures in the 125 mm. embryos. The retarded development of colloid in the ultimobranchial bodies in the pig corresponds with a similar retardation in its development in these structures in the Echidna in which, according to Maurer ('99), they remain independent structures. The time at which the transformation of the ultimobranchial bodies into typical thyroid structures is completed, that is, when they can no longer be distinguished from the derivatives of the median thyroid anlage, is variable. For example in a 175 mm. embryo their transformation is complete while in No. 2 of the full term embryos the left one is composed of an area of small follicles in which is located a small area of cell cords free from colloid (fig. 21). A comparison of the structure of the right ultimobranchial body, which is composed of an area of small follicles, and the left one in No. 2 of the full term embryos also shows that one ultimobranchial body may undergo a more rapid transformation into typical thyroid structures than the other in the same embryo.

Cell cords are formed from the periphery of the ultimobranchial bodies usually sooner than from their more central portion, as stated above. It is also in the cell cords of the peripheral portion of the ultimobranchial bodies that colloid is first formed, so that the older peripheral follicles of these structures in many stages are larger than the more centrally located ones. Figures 18, 19, and 21 show that the follicles containing colloid gradually decrease in size toward the more central portions of these structures. Since colloid appears first in the thyroid gland many of the follicles are quite large before colloid is first formed in the ultimobranchial bodies. It would thus seem that if the ingrowth of structural elements of the thyroid gland into the ultimobranchial bodies is a factor in breaking up the latter into cell cords,

as claimed by Simon, there would be some quite large thyroid follicles found in the deeper portion of the ultimobranchial bodies among the smaller ultimobranchial follicles which begin to develop comparatively late. However, excepting the cystoid follicles in the ultimobranchial bodies in some of the later stages, this condition is not found. The follicles containing colloid gradually increase in size from the more central portion to the periphery of these structures. It therefore seems that the contention of Simon is incorrect.

It also appears that in a few stages by far the larger portion of the ultimobranchial bodies undergo a transformation into typical thyroid structures even before colloid is formed in the thyroid gland. For example in embryos of 35 and 53 mm. in length the only structural features of the tripartite complex that can be interpreted as derivatives of the ultimobranchial bodies are small vacuolar areas (fig. 12) in contrast with the loosely arranged cell cords of these structures as found in embryos 37.5 and 60 mm. in length. Since in early stages it is impossible to distinguish the minute structure of the nonvacuolar portions of an ultimobranchial body from that of the thyroid gland when both are seen in the same microscopic field under high magnification, I believe that the vacuolar areas in embryos of 35 and 53 mm. in length represent only the more central cores of ultimobranchial bodies of which their more peripheral portion has undergone an early transformation into typical thyroid structures. This interpretation is supported by the conditions presented in a 48 mm. embryo in which the anterior portion of each ultimobranchial body is isolated from the thyroid gland. Excepting a small vacuolar area and traces of a lumen found in the isolated portion of the left one, the isolated portion of each of these bodies has a structure similar to the thyroid gland along which it lies.

I am of the opinion that the so variable developmental behavior of the ultimobranchial bodies in pig embryos throws light on a disputed point in connection with the development of these structures in human embryos. Grosser ('10) writes of a 'dichtere Zellgruppierung' in the thyroid gland of a human embryo

50 mm. long. He however does not believe that this dense cell area is derived from an ultimobranchial body but that it is "nur der Ausdruck intensivem Wachstums der ganzen anlage, während die Differenzierung der neugebildeten Stränge mehr oberflächlich stattfindet; die Zellen sind durchwegs typische Thyreoideazellen." Kingsbury ('14) finds that a human embryo 25 mm. long is the last stage in which the ultimobranchial body is clearly outlined. Their position in succeeding stages up to 41 mm. is occupied by a "poorly circumscribed area of denser tissue." He is of the opinion that this "inner condensation" "marks the place of disappearance of the ultimobranchial body although it may also well be as Grosser has stated, a center of growth." He further states that in 41 mm. and later developmental stages the "condensation is no longer recognizable." Although he was unable to satisfy himself as to the actual fate of the ultimobranchial bodies, he is of the opinion that they disappear.

From a study of the material used in this investigation I feel confident that the structure described by Grosser represents an ultimobranchial body. The process of cell cord formation at the periphery of the 'dichtere Zellgruppierung,' as described by him, corresponds favorably to the process of their formation in the ultimobranchial bodies in pig embryos. Both the 'inner condensation' (Kingsbury) and the 'dichtere Zellgruppierung' (Grosser) apparently represent the central core which in the ultimobranchial bodies of pig embryos is found in a very wide range of developmental stages, even in a full term embryo (fig. 21). It seems that if the 'dichtere Zellgruppierung' represented a proliferative center for the thyroid gland one would expect to find a rather large number of mitotic figures in them as an expression of rapid tissue growth. This, however, is not the case. No more nuclei in division are found in these areas than in any other portion of the thyroid gland.

The stages in which a comparatively early transformation of the greater portion of the ultimobranchial bodies takes place are comparatively few in number. Also there are comparatively few stages before full term in which there are no areas of small follicles

(ultimobranchial bodies) present. Judging, therefore, from the so variable developmental behavior of the ultimobranchial bodies it seems that the 175 mm. stage referred to above is one in which the ultimobranchial bodies underwent an early transformation into typical thyroid structures.

The portion of the structural elements of the thyroid gland at birth derived from the ultimobranchial bodies is small in comparison to the part derived from the median thyroid anlage. Owing to the variable developmental behavior of the former structures the comparative proportion contributed by them and the median thyroid anlage undoubtedly varies in different embryos. Figures 22 a, 22 b, and 22 c are diagrammatic representations of the portions derived from the median thyroid anlage and the ultimobranchial bodies in No. 2 of the 270 mm. (full term) embryos.

In the posterior portion of the right ultimobranchial body in No. 1 of the 125 mm. embryos is a cyst which extends through a series of sixty-seven sections (10 microns in thickness). It is lined with cuboidal epithelium the cytoplasm of which stained only very faintly. In one place in its lumen an isolated group of cells is found. The nature of its formation is unknown to me. According to Simon ('96) the formation of cysts in these structures is a regular occurrence during their 'période de survivance' in all animals examined by him, excepting in the pig in which they occurred in five out of eleven specimens. Since cyst formation occurred in only one specimen out of those I studied, it seems to be an exceptional developmental feature in the pig.

VI. CONCLUSIONS

1. The ultimobranchial bodies in the pig participate in the formation of thyroid follicles. However, the portion of the gland in full term embryos that is derived from these structures is small in comparison with the part derived from the median thyroid anlage.

2. The cephalo-caudal extent of the ultimobranchial bodies is equal to or nearly equal to that of the thyroid gland in embryos up to about 33 mm. in length. From this stage on to full term

the latter grows more rapidly in size than the former so that in stages from about 50 mm. in length to full term the ultimobranchial bodies usually lie in the posterior half of the thyroid gland but may be found in the middle third or in the middle two-fourths of the gland.

3. The developmental stages in which the ultimobranchial bodies transform into typical thyroid structures (that is, when they can no longer be recognized structurally from the median thyroid anlage), vary greatly. The transformation of the greater part of these structures may take place as early as in a 35 mm. stage, before colloid is present in the thyroid gland, but in the majority of stages examined it takes place in later stages. Even in full term embryos an entire ultimobranchial body may not be completely transformed.

4. The ultimobranchial bodies in a thyroid gland may vary in size, in shape, in the degree of their transformation, and in their location in the lateral halves of the thyroid gland. This variability is particularly pronounced in some of the later developmental stages.

5. Colloid first appears in the follicles of the thyroid gland in embryos of 75 mm. in length. A few small follicles containing colloid appeared first in the ultimobranchial bodies of a 125 mm. embryo. In a 145 mm. embryo the follicles containing colloid in these structures are quite numerous although on an average small in comparison with those in the thyroid gland.

6. Large cystoid follicles containing colloid may develop in the ultimobranchial bodies.

7. The ultimobranchial bodies usually become entirely imbedded in the thyroid gland. In a few developmental stages they were found to be only partially imbedded.

8. The formation of cysts in the ultimobranchial bodies of pig embryos is of rare occurrence.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

1 From a photograph of a transverse section of the ultimobranchial bodies and the thyroid gland about midway between the anterior and posterior ends of the latter. Before the fusion of the ultimobranchial bodies with the thyroid gland. From an embryo 18 mm. long. × 60.

2 a and 2 b From photographs of transverse sections of the ultimobranchial bodies and the thyroid gland taken respectively near the anterior and posterior ends of the latter. Fusion between the ultimobranchial bodies and the thyroid gland has in some places taken place. From an embryo 19.5 mm. long. × 60.

3 a, 3 b, and 3 c From photographs of transverse sections of the ultimobranchial bodies and the thyroid gland taken respectively near the anterior, middle, and posterior portions of the latter. The numerous deeply stained nuclei in the ultimobranchial bodies are represented by small black dots. From an embryo 20 mm. long. × 60.

4 From a photograph of a transverse section about midway between the two ends of the tripartite complex. The horns of the crescent are largely composed of the ultimobranchial bodies the left one of which is quite irregular along its dorso-mesial surface due to epithelial buds. From an embryo 21 mm. long. × 60.

5 From a photograph of a transverse section about midway between the two ends of the tripartite complex showing the large size of the ultimobranchial bodies. From an embryo 21.5 mm. long. × 60.

6 a and 6 b From photographs of transverse sections through near the middle and caudal portions respectively of the tripartite complex, showing numerous deeply stained nuclei (small black dots in the figures) in the ultimobranchial bodies and a few in the cell cords of the thyroid gland. The figures also show that the caudal portion of the ultimobranchial bodies are less broken up into cell cords than their more anterior portion. From an embryo 22 mm. long. × 60.

7 From a photograph of a portion of an ultimobranchial body showing degenerated and deeply stained nuclei. From an embryo 23 mm. long. × 650.

D.N., degenerated nuclei
D.S.N., deeply stained nuclei
Ep. B., epithelial buds
L., lumen
T., thyroid gland
Tr., trachea
U., ultimobranchial body

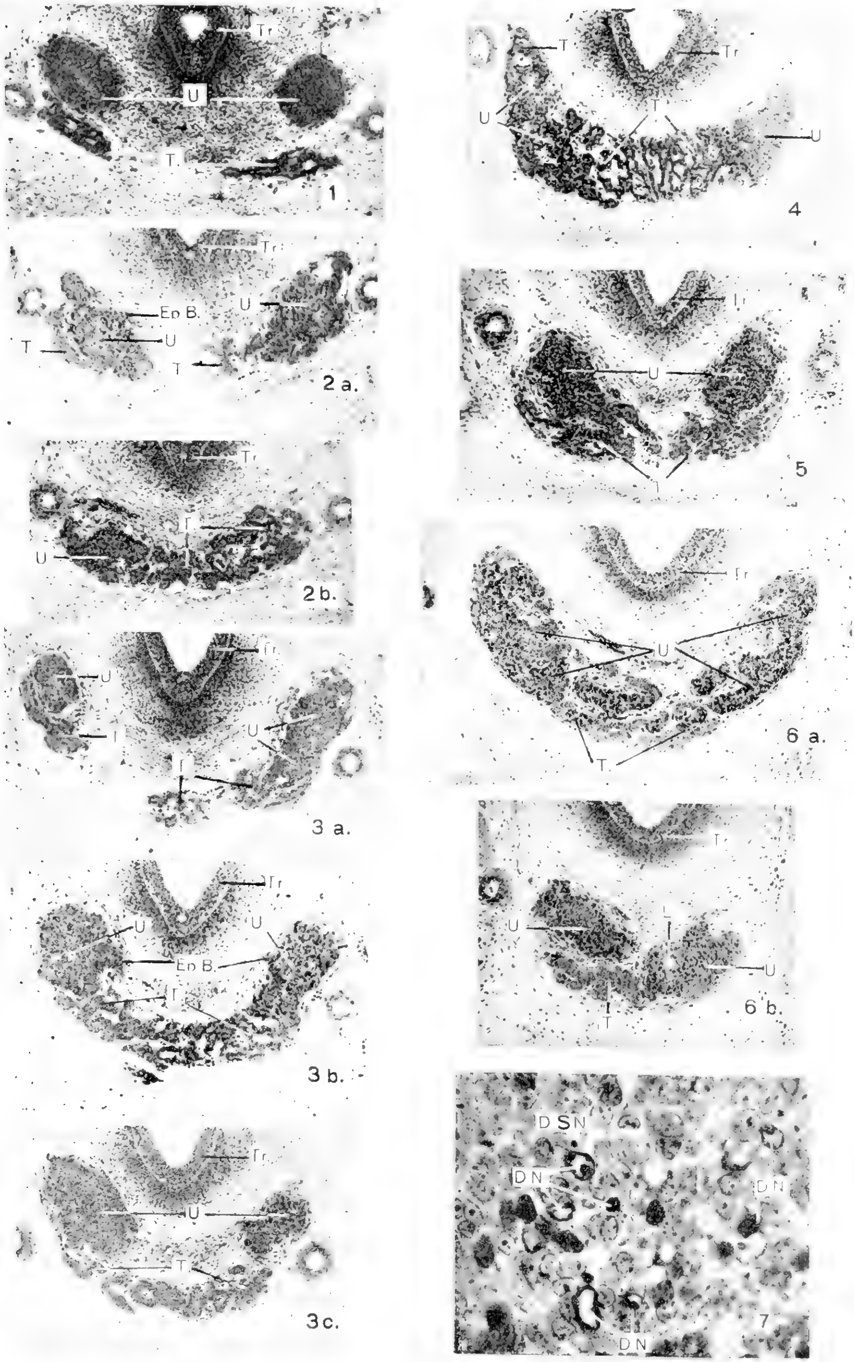

Tr
U
T
1
Ep B.
2 a.
2 b.
3 a.
3 b.
3 c.
4
5
6 a.
6 b.
DSN
DN
7

PLATE 2

EXPLANATION OF FIGURES

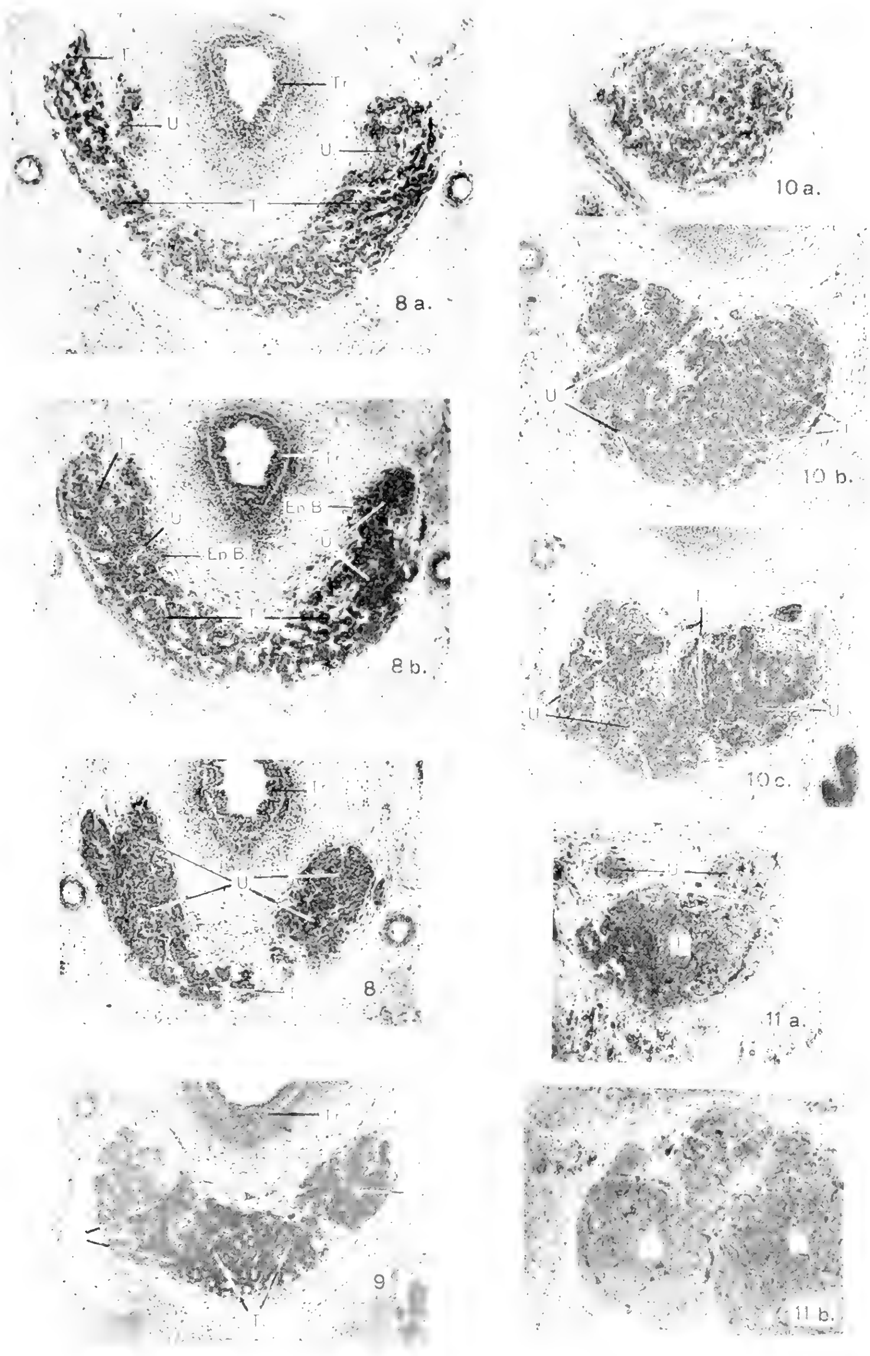
8 a, 8 b, and 8 c From photographs of transverse sections through near the anterior, middle, and posterior portions respectively of the tripartite complex, showing the gradual enlargement of the ultimobranchial bodies from their anterior to their posterior ends. The posterior end of the tripartite complex is largely composed of the ultimobranchial bodies. From an embryo 23 mm. long. × 60.

9 From a photograph of a transverse section about midway between the two ends of the tripartite complex showing the ultimobranchial bodles largely broken up into coarse cell cords. From an embryo 27 mm. long. × 56.

10 a, 10 b, and 10 c From photographs of transverse sections through the anterior, middle and posterior portions respectively of the tripartite complex. The ultimobranchial body on the left side extends along the posterior three-fourths of the thyroid gland while the right one along only its posterior fourth. The unequal size of the two ultimobranchial bodies, which are largely broken up into coarse cell cords, produce the asymmetry of the tripartite complex. From an embryo 29.5 mm. long. × 56.

11 a and 11 b From photographs of sections through the anterior and nearly the middle portions respectively of the tripartite complex showing both ultimobranchial bodies separated from the anterior portion of the thyroid gland (fig. 11 a) and the left one with traces of the lumen also separated from the thyroid (fig. 11 b). From an embryo 48 mm. long. × 60.

Ep.B., epithelial buds
L., lumen
T., thyroid gland
Tr., trachea
U., ultimobranchial body


T
Tr
U
U
T
8 a.
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En B
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En B
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8 b.
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Tr
T
9
10 a.
U
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10 b.
T
U
U
10 c.
U
T
11 a.
11 b.

PLATE 3

EXPLANATION OF FIGURES

12 From a photograph of a small portion of a section through an ultimobranchial body (the light area in the figure) which merges gradually into typical thyroid structures. In the light area the majority of the ultimobranchial nuclei do not stain deeply. From an embryo 53 mm. long. × 337.

13 From a photograph of a portion of a transverse section of the tripartite complex showing the right ultimobranchial body which is largely composed of coarse and loosely arranged cell cords. From an embryo 60 mm. long. × 60.

14 From a photograph of a portion of a section of the tripartite complex showing the right ultimobranchial body which, in this particular place, is composed of loosely arranged cell cords in which no colloid is present. From an embryo 100 mm. long. × 56.

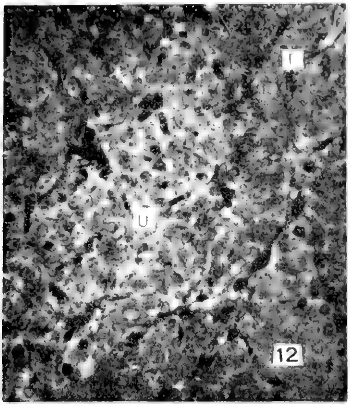
15 From a photograph of a portion of a transverse section through the posterior portion of the tripartite complex showing the compactly arranged cell cords of the right ultimobranchial body in which is located a cyst. The area inside the dotted circle is free from colloid. From an embryo 125 mm. long. × 45.

16 a and 16 b From photographs of portions of a transverse section of the tripartite complex showing respectively the right and left ultimobranchial bodies. The right one is only partially imbedded in the thyroid gland and contains many cystoid follicles (*C.F.*) which do not contain colloid and a few small follicles which contain colloid (*Co*). The black dots in the portion of the figure labeled 'thyroid' represent colloid. The left ultimobranchial body is more deeply imbedded in the thyroid gland. From an embryo 125 mm. long. × 38.

17 From a photograph of a portion of a transverse section of the tripartite complex showing the right ultimobranchial body in which are found both small and cystoid follicles that contain colloid. From an embryo 145 mm. long. × 38.

18 From a photograph of a portion of a section of the tripartite complex showing the left ultimobranchial body which is represented by an area of small follicles. The black dots in the figure represent colloid. From an embryo 160 mm. long. × 38.

C., cyst
C.T., cystoid follicles
Co., colloid
T., thyroid gland
U., ultimobranchial body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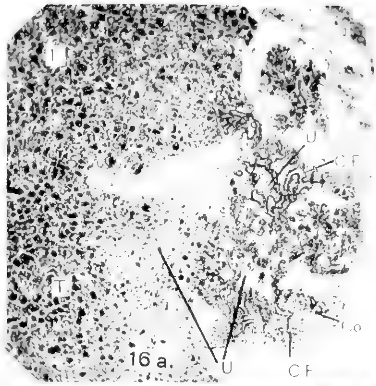
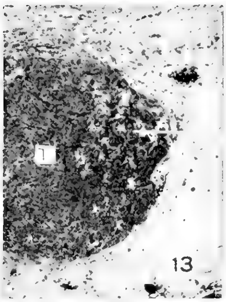
12


T
U
C.F.
T
Co
16 a.
U
C.F.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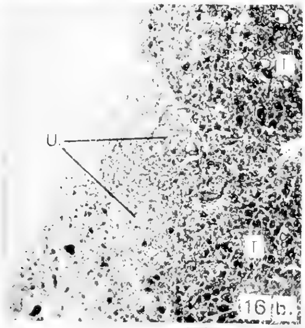
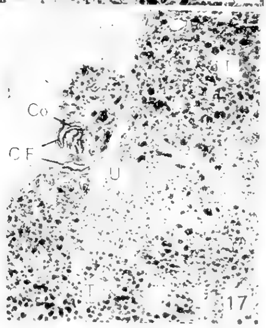

T
U.
T
16 b.


U
T
14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C.F.
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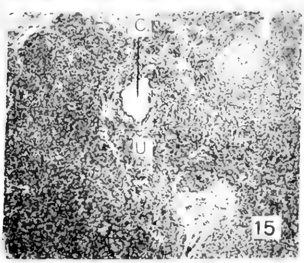
C.
U
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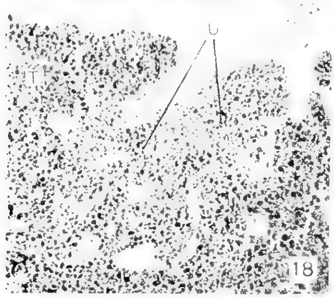

U
T
18

PLATE 4

EXPLANATION OF FIGURES

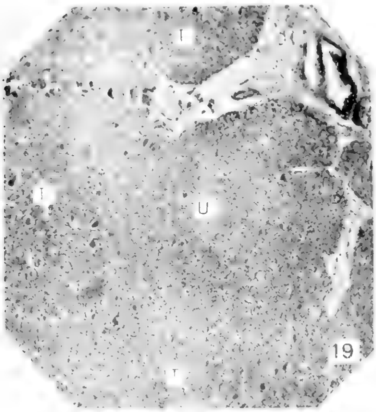
19 From a photograph of a portion of a section through the left ultimobranchial body and a portion of the thyroid gland surrounding it. The ultimobranchial body is characterized by follicles which contain colloid and which are on an average appreciably smaller than the follicles of the thyroid gland. From an embryo 225 mm. long. × 38.

20 From a photograph of a portion of a section through the right ultimobranchial body and a portion of the thyroid gland surrounding it. The ultimobranchial body contains many cystoid follicles which contain colloid. The colloid dropped out from some of the follicles during the process of staining. From an embryo 245 mm. long. × 38.

21 From a photograph of a portion of a section through the left ultimobranchial body and a portion of the thyroid gland. The ultimobranchial body is characterized by an area of small follicles in which is located a small area free from colloid. The light dots represent follicles from which the colloid has fallen. This figure represents the ultimobranchial body at *C* in figure 22 a. From No. 2 of the embryos 270 mm. long (full term). × 38.

22 a, 22 b, and 22 c These figures show the relative size of the ultimobranchial bodies and the thyroid gland in No. 2 of the embryos 270 mm. long (full term). The extent of the ultimobranchial bodies is outlined by a dotted line. Inside the left ultimobranchial body is a small area (X), also outlined by a dotted line, which is free from colloid (figs. 22 a and 22 c). The portion of the left ultimobranchial body outside the area X and all of the right one is characterized by follicles which are on an average appreciably smaller than those of the thyroid gland. Figures 22 b and 22 c represent cross sections through the tripartite complex at b and c respectively of the structures represented in figure 22 a. × 7.5.

T., thyroid *U.*, ultimobranchial body


T
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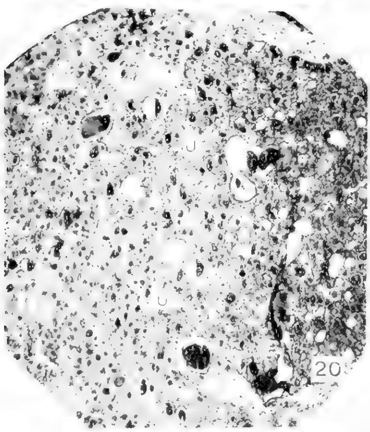
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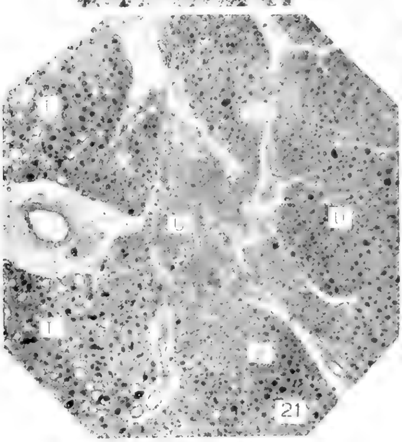
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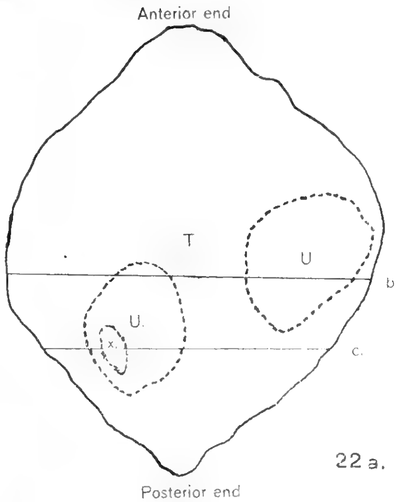
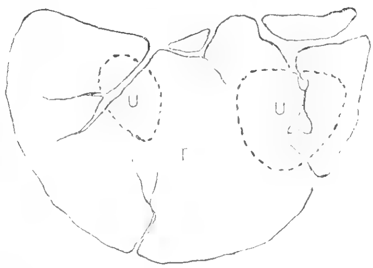

Anterior end
T
U
U.
x
b
c.
Posterior end

22 a.


U
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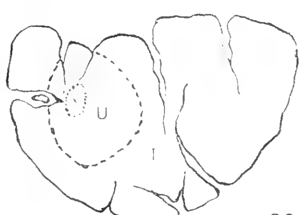
22 b.


U
T

22 c.

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CHONDRIOSOMES IN THE TESTICLE-CELLS OF FUNDULUS

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TWENTY-ONE FIGURES (TWO PLATES)

Our knowledge of chondriosomes in the spermatogenesis of fishes is limited, as far as I know, to an incomplete account on Myxynoids by A. and K. E. Schreiner ('05, '08). In the ripe spermatozoon, however, the same bodies have been studied, especially by Retzius, in quite a large number of species.

According to A. and K. E. Schreiner, the chondriosomes are represented in the spermatogonia as well as in the spermatocytes of Myxine glutinosa by very small granules, tightly crowded together in the neighborhood of the 'Sphäre.' No change in shape is observed during mitosis; furthermore, the behavior of the mass of mitochondria seems to be entirely passive and consequently its segregation between the daughter-cells is often unequal. Concerning the process of spermiogenesis, these authors merely state that the mitochondria build a sheath around the axile filament. It must be added that the preservation of the chondriosomes in the material used by A. and K. E. Schreiner can hardly be considered as satisfactory.

Retzius has studied the ripe spermatozoon of Amphioxus ('05 b), of several selachians ('09 c; '10 b), of one ganoid (Amia calva, '05 b) and of a number of teleosts ('05 b; '10 b). As data concerning the process of spermiogenesis in selachians are lacking, in reference to the chondriosomes at least, it is hardly possible to decide what part of the spermatozoon is formed by these bodies. In the other classes however, their identification is easier and the concordant observations of Retzius on Amphioxus, Amia and teleosts can be summarized as follows: the chondriosomes of the ripe spermatozoon are located at the posterior part of the head

and surround usually for a short distance the proximal part of the tail. The shape of this sheath varies with the different species. In Amphioxus the chondriosomes are represented by a rather voluminous body in which, by careful study, one can make out three to five granules. In Amia, such a body appears indistinctly granular and fits the posterior part of the head as the cup fits the acorn. In teleosts similar dispositions are found, for the details of which I refer to Retzius' papers. I wish to emphasize that in a number of species the granules are very distinct and even constant in number. In Lophius piscatorius for instance, Retzius ('10 b) invariably found four of them, disposed in regular order around the origin of the tail.

It may well be recalled that a similar disposition of the chondriosomes has been observed in invertebrates. For instance, according to the observations of Meves ('00, '03), each spermatid of the apyrene generation of Paludina vivipara contains four chondriosomes. They assume the form of spheres and occupy the posterior part of the head, where they surround the axile filament. Bonnevie ('07) gives a similar description for Membranipora pilosa. In these cases however, this stage is a transitory one, for the shape of the chondriosomes changes during the further evolution of the spermatid, while in other invertebrates the same arrangement is, according to Retzius, retained in the ripe spermatozoon, namely, in a number of celenterates ('04 a and b; '05 a; '09 a), in many echinoderms ('04 a and b; '05 a; '10 a),[1] in worms ('04 a and b; '05 a; '06 b, c and d; '09 b) and in molluscs ('04 a and b; '05 b; '06 a; '10 b). In many species belonging to the two last-named classes the numerical constancy of the chondriosomal spheres and the regularity of their arrangement around the axile filament are conspicuous features of the ripe spermatozoon. Especially remarkable is the disposition in

[1] Meves ('12) contests the accuracy of Retzius' description for Parechinus miliaris. He finds that the so-called 'Mittelstück' is not granular, as stated by Retzius, but homogeneous, and that it has the shape of a ring, through which runs the axile filament. I take this opportunity to remind how inadequate is the expression 'Mittelstück' or 'middlepiece,' as, between the 'Mittelstück' of the spermatozoon of an echinoderm, of a selachian or a urodele amphibian and of a mammal, there is no homology whatever.

polychetes: the granules in many species are consistently four in number, their form being exactly spherical, their volume equal and their disposition around the proximal part of the tail perfectly regular.

The origin of these bodies is briefly referred to by Retzius ('04 a and b) who states (for molluscs) that the large spheres are formed by the confluence of smaller granules. Previously, Pictet ('91) and Field ('95) both had described the formation of the so-called 'mittelstück' in the spermatozoon of echinoderms through fusion of highly refractive granules which, however, they erroneously derived from remnants of the spindle.

Quite recently M. R. Lewis ('17) has stained the chondriosomes (the so-called 'middlepiece') of the spermatozoon of Cerebratulus lacteus and of Echinorachnius parma in vivo, by using a solution of Janus-green in sea-water.[2]

The object of the present investigation is the testicle of Fundulus (heteroclitus and majalis), the main purpose being to study the behavior of the chondriosomes during spermatogenesis. The material was collected in Woods Hole, Mass., in June, 1916, and fixed in Regaud's or in Benda's fluid, the latter either with or without acetic acid. The sections, 5 μ thick, were stained in the first case with iron-haematoxylin or acid fuchsin-methylgreen; after Benda's fixation, I resorted to iron-haematoxylin acid, fuchsin-methylgreen or Benda's stain, the latter giving, as previously stated for embryos ('17), a very small percentage of good preparations. A number of sections were stained with safranin, in order to study the chromatin.

Once more I found that the preservation of the tissue is much better after Benda's fixation than after Regaud's. This last reagent has a pronounced tendency to make the seminal cells

[2] In the same paper, M. R. Lewis (p. 33) quotes my opinion, as expressed in my review ('12), on the vital staining of chondriosomes and, from this quotation, one might be induced to conclude that, for me, neutral red and methylenblue can be used or have been used to stain the chondriosomes in vivo. To prevent any misunderstanding, I wish to recall that this has never been my opinion, as appears clearly in the quoted place of my article (p. 608), as well as in several others (for example, page 823, in the discussion of Arnold's plasmosomes).

swell. The extent to which the ground substance is affected is well illustrated by the difference in size exhibited by the cells represented in figures 10 and 11, both in exactly the same stage of evolution, the first from material fixed in Benda's fluid, the second from material treated with Regaud's. Thus, cells which normally float freely in the cystic cavity are made to conglomerate and stick together. The chondriosomes are also swollen, and the chromosomes are transformed into an undecipherable clump. In contrast to this, the last-named bodies are well preserved in Benda's material, an appearance which confirms that swelling in Regaud's rather than shrinking in Benda's fluid is responsible for the differences between the two sets of preparations.

The testicle of Fundulus is at the time of spawning a rather voluminous organ formed by a considerable number of tubular cysts in which spermatogenesis proceeds from the periphery towards the hilus.[3] The excretory system of the gland consists of a number of ducts lined with cubic or cylindric epithelium. In the distal part of these ducts the cells (fig. 1) contain, besides secretion-granules, a large number of chondriosomes. These are mostly long chondrioconts running along the nucleus in a direction perpendicular to the basis of the cell and intertwining at both poles of the nucleus. This disposition reminds one somewhat of the structure of the cells of the tubuli contorti (Heidenhain's rods) or of the salivary ducts (Pflüger's rods). The inner part of the cell is often free of chondriosomes and irregularly delimited, an appearance which may be due to the action of the fixing fluid.

In the cells lining the proximal part of the excretory ducts, the chondriosomes are all replaced by granules of pigment. This recalls an observation made by Prenant ('11) on the skin and cornea of the frog. Prenant found that the cells of both layers in the skin contain mitochondria and pigment-granules. In the upper layer the granules of pigment are located near the surface, the mitochondria in the lower part of the cell, while in the deeper layer mitochondria and pigment-granules are mixed

[3] Degenerating cells are, as in other testicles and especially in invertebrates, by no means infrequent in Fundulus.

together. In the cornea no pigment is present. If one studies the point of transition between cornea and skin, one can see how the mitochondria gradually take the place of the pigment-granules. This observation is interpreted by Prenant, apparently not without reason, as indicating the transformation of chondriosomes into pigment and in the same sense could be interpreted the conditions just described in the excretory ducts of the fish-testicle.

The seminiferous cysts are reunited by thin sheets of connective tissues containing blood-vessels and cells. Some of these are conspicuous by their large size and by the presence of a great number of bacilli-shaped chondrioconts (fig. 2); others contain also granules which I am inclined to consider as secretion-products. In places where the connective tissue is somewhat more abundant, for instance in such stellar spaces as appear between the cross sections of the cysts, they usually build groups of two or more elements. The nearest interpretation of these cells is that they correspond to the interstitial cells of the mammalian testicle. Supposing I were right, this would be the first mention of them in fishes, for, as far as I know, the literature does not contain any mention of interstitial tissue in this class of vertebrates: in fact Friedmann ('98) and Ganfini ('02) state positively that they could not find it.

The distal part of the cysts is occupied by cells which are obviously the stem of the whole seminal lineage and as such should be designated as spermatogonia. Since, as we shall see, several generations of spermatogonia can be distinguished, I would call these 'primary spermatogonia.' Their size is relatively large (fig. 3, two cells on the top row and two cells at the right). Each nucleus contains usually only one large, sharply delimited, spherical block of chromatin. The eventual occurrence of multiple nucleoli is often accompanied by the presence of indentations (the process is just indicated in figure 3, in the cell of the top row, to the right), which are suggestive of direct division. Mitosis however was repeatedly observed (figs. 4 and 5). It would not be surprising if these indentations were indicative of a process described as occurring in the spermatogonia of

Salamandra after the period of sexual activity (namely by Meves '91), as once that period over, the testicular conditions are very similar in both the amphibian and the fish.

The chondriosomes of the primary spermatogonia deserve special mention. In the resting cell they are numerous, coarse and irregular granules or rods. Most of them are located very close to the nucleus and cover its surface. This disposition might be interpreted in favor of Goldschmidt's chromidial theory. Such a claim however would be unfounded: Goldschmidt and his pupils basing themselves upon defective observations, expected to demonstrate that the chondriosomes of the germ-cells were formed during the growth-period and they have failed utterly.[4] The continuity of the chondriosomes on the other hand has been demonstrated in a number of animals and is strongly supported for fishes by my observations on the fish-embryo ('17).[5] It is however far from my mind to deny the

[4] For a complete historical and critical account of the chromidial theory, see the third chapter of my review ('12). Shaffer, who seems inclined to believe (p. 414) in a nuclear origin of the chondriosomes, gives as an argument that "in nearly all the growth-stages of the first spermatocytes, there is present a denser and more deeply staining perinuclear zone," formed by the chondriosomes. I should take exception to this statement, for it is characteristic, even if not quite general, that the male auxocytes have their chondriosomes accumulated at one pole of the nucleus, around the idiozome.

[5] In a paper on the testicle of opossum, Jordan ('11) claims that he has demonstrated the discontinuity of the chondriosomes in the seminal cells. I have been investigating lately the same object and my observations are in direct contradiction with Jordan's claim: chondriosomes exist in abundance in all the stages of the evolution of the seminal cells.

Shaffer ('17) enters against the theory of the continuity of the chondriosomes in the following way: "(p. 423) the progressive increase in the amount of mitochondria (during the evolution of the seminal cells) seems to indicate that they are differentiation-products. Hence, if there is any genetic continuity between the mitochondria of successive cell-generations, it is only of a limited sort. The conception that the mitochondria present in the somatic cells are the direct descendants of those of the germ-cells, from which they have arisen, certainly has very little evidence in its favor." I must state that I entirely fail to see an argument against the continuity of the chondriosomes in the fact that their amount may increase. Concerning the continuity of the chondriosomes in the somatic cells with those of the germ-cells, Shaffer overlooks apparently the numerous observations which have shown this continuity, from the egg at least to the embryonic cells. I limit myself to remind of my own observations on the bee,

existence of nucleocytoplasmic exchanges, as the nucleus is certainly not a sort of impermeable rubber-vesicle enclosed in the cell. But it would be rash to base on the mere existence of such appearances as described above any definite conclusion. The arguments for the cytoplasmic nature of the chondriosomes I do not want to repeat here and refer the reader to former papers, limiting myself to state that no indications of a nuclear origin can be found in the staining reactions.[6]

During the mitotic division of the primary spermatogonia the shape of the chondriosomes changes somewhat: they round up and become more regular (figs. 4 and 5). Their location in the cell is also modified: at the stage of metaphase they surround the spindle (fig. 4) and later are found between the daughter-nuclei (fig. 5).[7]

Next to these cells are others differing but slightly from them. They are somewhat smaller in size and their chondriosomes are not quite so coarse. These cells are assembled in rosettes of

the rabbit and quite lately on Ciona, where the chondriosomes form the material of the yellow crescent, the continuity of which has been demonstrated by Conklin.

[6] The original colors of the preparations could not be reproduced in the plates; as is well known, they are, in acid fuchsin-methylgreen preparations, red for the chondriosomes and green for the chromatin; in Benda's preparations, dark purple for the chondriosomes and pale brown for the chromatin.

[7] Concerning the fate of the chondriosomes during the mitotic division of the spermatogonia of Passalus, Shaffer expresses himself as follows (p. 410): "the spermatogonial cysts which are in mitotic activity, stand out very clearly in contrast with the resting cysts. This is because of their lighter staining capacity; whether this in turn is due to the partial disappearance of the mitochondria could not be ascertained." Shaffer quotes Buchner as having found that in Gryllotalpa vulgaris, the chondriosomes disappear during or just before cell-division and gives three possible explanations "for the partial loss of mitochondrial structure during mitotic activity." Interesting though they may be, these explanations appear to me for the present useless, as, after my own experience, chondriosomes do not disappear during mitosis, no more in Gryllotalpa, as I have shown ('10), than in any other case I know of.

Payne ('17) quotes both Buchner and me and sees no reason why we should differ so much in our observations: "In this case, one or the other has certainly made a mistake." Between a negative result, however, and a positive one, there is, in my opinion, no room for hesitation. It must be added that since, Buchner has considerably modified his attitude towards the chromidial theory, as appears from a text-book he recently published.

three or more (fig. 3 on the left below). So unvarying are these features that I feel justified in considering these cells as a distinct generation of spermatogonia and term them 'secondary spermatogonia.' The primary and secondary spermatogonia are in close contact with each other, the cystic cavity being at these stages only virtual, in contrast with all later stages, when some room, in well fixed material, is left between the cells.

The spermatogonia belonging to a third generation are, if any, not much smaller than the secondary spermatogonia. In the nucleus several blocks of chromatin are present. The chondriosomes are granules, most of them regular, some larger and coarser. Instead of surrounding the nucleus, as in the preceding generations, they are all located at one of its poles (fig. 6). During mitosis a breaking-up into smaller granules appears to take place. Their behavior is the same as described above and is illustrated for the stage of metaphase by figure 7. In fact, the size of the spindle is in proportion to the size of the cell so large that the chondriosomes have to take whatever place they can in the cell-body, which is practically filled by the karyokinetic figure.

In the first spermatocytes (fig. 8) the polar location of the chondriosomes persists throughout the whole growth-period until the prophase of the first division and coincides as always with the polar field, while in the nucleus the usual structural changes take place. The chondriosomes are now granules all equal in size and regularly spherical and most of them are very closely heaped together. It must be noted that during this so-called growth-period the spermatocytes of Fundulus actually grow very little and that there is no evidence, as in other spermatocytes, of an increase in the mass of chondriosomes.

At the prophase of the first division the mitochondria become scattered all around the nucleus and, when the spindle is formed, they are as previously pushed towards the periphery of the cell-body and very close to it; for here again the spindle is very large in proportion to the cell. I may mention in passing that the centrioles appear very conspicuously at the poles of the spindle (fig. 9). During the anaphase all the mitochondria are found

between the daughter-nuclei (figs. 10 and 11). The same process is repeated during the second division (fig. 12). Though the cells are very small, it is easy enough to distinguish both mitoses owing to the following characteristics. The first spermatocytes are larger than the second ones. The spindle at the stage of metaphase is more slender in the second division. The number of chondriosomes decreases conspicuously. Finally the size and shape of the chromosomes as observed in Benda's material present a most distinctive character: in the first division they are unmistakably heterotypic.

The spermatids, which are exceedingly small, very soon form an axile filament. At first the mitochondria are scattered all around the nucleus but only for a short time. In the succeeding stage which is very characteristic and which, judging from its frequent occurrence in the preparations, lasts apparently a considerable period, all the mitochondria are found accumulated in one heap at the posterior pole of the nucleus where they surround the proximal part of the axile filament (fig. 13). A glance at these cells readily gives the impression that the number of their mitochondria is constant. When one attempts to count them however, one realizes that to obtain exact figures is almost impossible, for the granules are very small and not all in the same level. The numbers I found in the most favorable cases came very close to eight.

Further stages of spermiogenesis are characterized by changes in the mitochondria (which will be described below), the growth of the tail and the following modifications of the nucleus. First, the posterior side, which is in close contact with the mitochondria, becomes flattened or even somewhat concave (fig. 14). Its chromatic content then gradually accumulates at the periphery, with the exception of the posterior or flattened side, a process whose occurrence has been described several times in invertebrates and which begins in Fundulus at the stage represented by figure 14. The crust of chromatin thus formed assumes the outline of a horse-shoe, the space existing between the free ends of its branches being occupied by the mitochondria; from the same space emerges the axile filament (fig. 15 et seq.). Later,

the head becomes somewhat elongated and the branches of the horse-shoe are by the same process brought nearer together (fig. 16, 17 and 18). At the same time the head loses its symmetry inasmuch as it becomes somewhat curved along its antero-posterior axis and its posterior facet becomes oblique, instead of being perpendicular, to the same axis. From this time on we can distinguish what I have, arbitrarily of course, termed face-views (figs. 16 and 21) and side-views (figs. 17, 18 and 20) of the spermatozoon.

All the modifications of the head are more easily followed on acid fuchsin-methylgreen preparations than on Benda's, for methylgreen gives a sharper stain for chromatin than sodium-sulfalizarinate. In material fixed with Regaud's fluid the clear middle-space of the head appears very conspicuous even in the last stages; but curiously enough, as soon as the spermatozoa have reached the excretory ducts, the staining reaction changes and the head takes up acid fuchsin instead of methylgreen. In preparations made from material fixed with Benda's fluid the ripe spermatozoa, that is, those which have reached the excretory ducts, appear somewhat different from those fixed in Regaud's fluid. In a side-view (fig. 20) the clear middle-space appears only indistinctly. In face-views (fig. 21) on the other hand, the same space is very conspicuous and sharply delimited, and has the appearance of a canal running from the posterior to the anterior extremity of the head.

During this period changes take place in the mitochondria also. Their number decreases and their size increases: in other words, there is a fusion of granules. This process can be best followed in Regaud's preparations for the reason that the thin sheet of protoplasm which keeps the mitochondria in place (figs. 14 and 15) and which is hardly visible in Benda's preparations, swells in Regaud's fluid as do also the mitochondria themselves. Consequently, the cells and the granules are somewhat larger than in Benda's preparations and they are more scattered. These differences are well illustrated by figures 15 and 19, which represent approximately the same stage, after Regaud's and Benda's fixation respectively. Thus in figure 15 we can count

exactly six granules while Benda's preparations of the same stage (fig. 19) show an undecipherable heap of mitochondria. Later when the asymmetry of the head has become conspicuous, we find almost invariably four mitochondria (fig. 16), disposed with remarkable regularity upon the posterior facet of the head. Finally in the ripe spermatozoon the number is still more reduced, usually to three. Here Benda's material is more serviceable than Regaud's owing to the change in the staining reactions of the head mentioned above. A comparison of the different stages of this evolution, as they appear after fixation in Regaud fluid, shows that the increase in volume of the mitochondria is not directly proportional to their decrease in number (figs. 13 to 18); and, as there is no evidence of an elimination of mitochondria, one would be led to believe in a strong condensation of the chondriosomal substance. This conclusion is however not supported by Benda's preparations and I am forced to admit that the swelling produced by the formalin-bichromate mixture is greater in the first stages of spermiogenesis than in the later ones.

As stated above, the average number of mitochondria in the ripe spermatozoon, as counted in Benda's preparations, is three. They are especially conspicuous in face-views (fig. 21), where they are found regularly disposed on the posterior facet of the head. Occasionally spermatozoa are found with four, five or even six granules taking the chondriosomal stain. The majority of these granules are undoubtedly mitochondria and in such cases the fusion has, for some unknown reason, apparently not proceeded normally. Whether it is completed later is difficult to say. It is probable also that occasionally the centrioles are stained, for in certain cases it was possible to recognize a relationship between the proximal extremity of the axile filament and a small granule stained like a chondriosome (fig. 20). I cannot give any definite information about the behavior of the centrioles during the spermiogenesis of Fundulus,[3] but there is no doubt that they are located in that region.

[3] One thing however is certain: that their behavior is very different from the same in selachians (Suzuki, '98).

Again as in many and perhaps all cases, the last stages of spermiogenesis bring about a change in the behavior of the chondriosomes towards reagents. It is well known that, in the mammalian testicle for example, the chondriosomes become more and more resistant to acetic acid as spermiogenesis progresses.[9] The test of this resistance was not made here, but it was found that the chondriosomes of the last stages are structures much less labile than the chondriosomes of the early stages and are consequently much easier to bring into evidence.

The preceding description of the spermatozoon of Fundulus agrees in the main with Retzius' observations on the spermatozoon of other teleosts, though differing in the details. It helps at the same time to emphasize the similarity in structure between these spermatozoa and those of a large number of invertebrates, while the spermatozoa of selachians and of the higher vertebrates are widely different.

From the same description it also appears very probable that the male chondriosomes, owing to their close contact with the nucleus, are carried into the egg at the time of fertilization. Though this can be ascertained only by the study of the fertilizing process, the evidence accumulated by an imposing number of observations made upon almost all classes of animals, especially in recent years, is certainly very much in favor of the theory according to which the penetration of the male chondriosomes into the egg is a general phenomenon. Shaffer who mentions only Meves' observations on Ascaris and Vander Stricht's on the bat, overlooks the largest part of this evidence. That Lillie ('12) found in Nereis that the 'middle-piece' and the tail of the spermatozoon do not enter the egg does not prove that the chondriosomes are not carried into it.

I still believe, as in 1915, that the real objection to the admission that the male chondriosomes play a rôle in heredity is to be found in Meves' observations on the echinoderm-embryo; why their admitted chemical composition should plead against

[9] I found recently that the same changes take place in the spermatids of opossum.

such a rôle, as Cowdry ('16, p. 437) seems to believe, I fail entirely to see. Concerning the hypothesis of their motile function which, first formulated by Benda, reappears occasionally in the literature, I do not see that any arguments have been brought forward in its favor, nor is there any clear expression of how we should imagine this function. Benda considered his 'mitochondria' as contractile bodies: how can this conception be applied to the spherical chondriosomes of the spermatozoa of so many invertebrates and of Fundulus? Furthermore, those who advocate this hypothesis entirely overlook two groups of observations, which we have to accept as long as their inexactitude has not been demonstrated: first, Meves' experiments on the spermatozoon of Salamandra and second, the observations of a number of authors, lately Koltzoff's, on the spermatozoa of decapods (see Duesberg, '12, p. 687).

Finally a few words concerning the occurrence of a constant number of chondriosomes in male germ-cells.

The first indication of this was given by Meves ('00) who found that the small spermatocytes (i.e., as the apyrene generation) of Paludina vivipara contain on the average eight loop-shaped chondriosomes. Numerations made on spermatids of the same generation a short time after the second division likewise revealed an almost unvarying number of chondriosomes, this time four.

Two other cases, much more striking, have been described lately, both in arachnoids, the first one by Sokolov ('13), the other by Wilson ('16).

In the spermatogonia and in the young spermatocytes of Euscorpius carpathicus Sokolov describes mitochondria which soon by confluence form filaments. Later rings appear, which are probably formed by fusion of the free ends of the filaments of the preceding stages. The average number of these rings is twenty-four. During mitosis they are not divided as is the case in the small spermatocytes of Paludina, but are segregated into two equal groups between the daughter-cells. Thus each spermatid contains one quarter of the number of rings, on the average six.

The result of this process is an obvious and measureable reduction of the chondriosomal mass at the end of the divisions of maturation and Sokolov sees in it a confirmation of the views I have expressed as the result of my study of the behavior of the chondriosomes in the spermatocyte-divisions of the rat ('07).

Wilson has studied the chondriosomes in the spermatogenesis of two other species of scorpions, Opisthacanthus elatus (Southern California) and Centrurus oxilicauda (Southern Arizona). The results obtained from the study of the first named species are very similar to those of Sokolov. Each spermatocyte contains about twenty-four hollow spheroidal bodies, which are segregated by the spermatocyte-divisions into four approximately equal groups. Each spermatid thus receives as a rule six chondriosomes (in 73 per cent of the cases, on 200 numerations), sometimes five (in 16 per cent of the cases) or seven. No other numbers were observed. In the Arizona-scorpion, the process is quite different. All the chondriosomal material becomes concentrated in a single definite body in the form of a ring. This ring divides during mitosis in such a way that each spermatid receives exactly one-fourth of its substance, "the process taking place with a precision that is comparable to that seen in the distribution of the chromosome material."

As Wilson points out the body in question represents a hitherto undescribed type of chondriosome. The occurrence of this interesting process makes one speculate as to what the field of spermatogenesis, though so widely explored, still has in store for the investigator. It appears to me that conditions similar to those found in scorpions, at least to those found in Euscorpius and in Opisthacanthus, could be expected in the histogenesis of these spermatozoa in which, as stated above, the chondriosomes are represented by a constant or approximately constant number of well-defined granules. There is some indication of a similar process in Fundulus, but the small size of the cells unfortunately makes an exact numeration impossible. The same difficulty would certainly be met with in the study of the seminal cells of other teleosts as well as of echinoderms and celenterates; molluscs and worms, however, would probably be a favorable material.

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PLATES

EXPLANATION OF FIGURES

All figures were outlined with a Zeiss camera-lucida, at the level of the stage of the microscope. Lens used: Zeiss apochr. imm. 1 m.m., 5; ocular 12. Artificial light (gas).

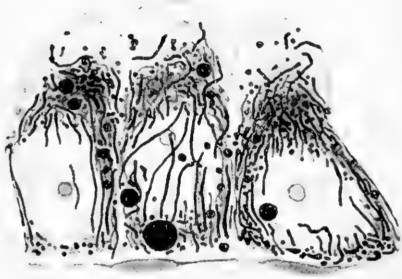
PLATE 1

EXPLANATION OF FIGURES

1 Fundulus majalis. Fixation: Benda, without acetic acid. Stain: Benda. Epithelium of an excretory duct.

2 Fundulus heteroclitus. Fixation: Benda. Stain: Benda. Supposed interstitial cells.

3 Fundulus majalis. Fixation: Benda, without acetic acid. Stain: Benda. Group of primary and secondary spermatogonia.



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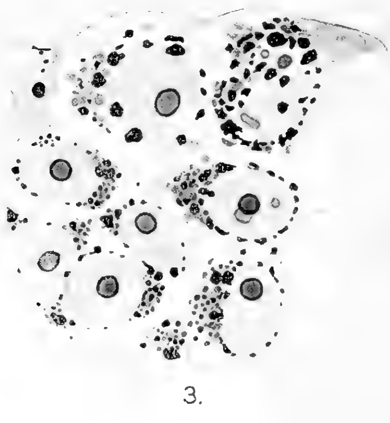


3.

PLATE 2

EXPLANATION OF FIGURES

4 and 5 Fundulus heteroclitus. Fixation: Benda. Stain: Benda. Metaphase and anaphase of the mitotic division of primary spermatogonia.

6 Same material. Tertiary spermatogonium.

7 Same material. Tertiary spermatogonium: metaphase.

8 Fundulus majalis. Fixation: Benda, without acetic acid. Stain: Benda. First spermatocyte.

9 and 10 Same material. Metaphase and anaphase of first division of maturation.

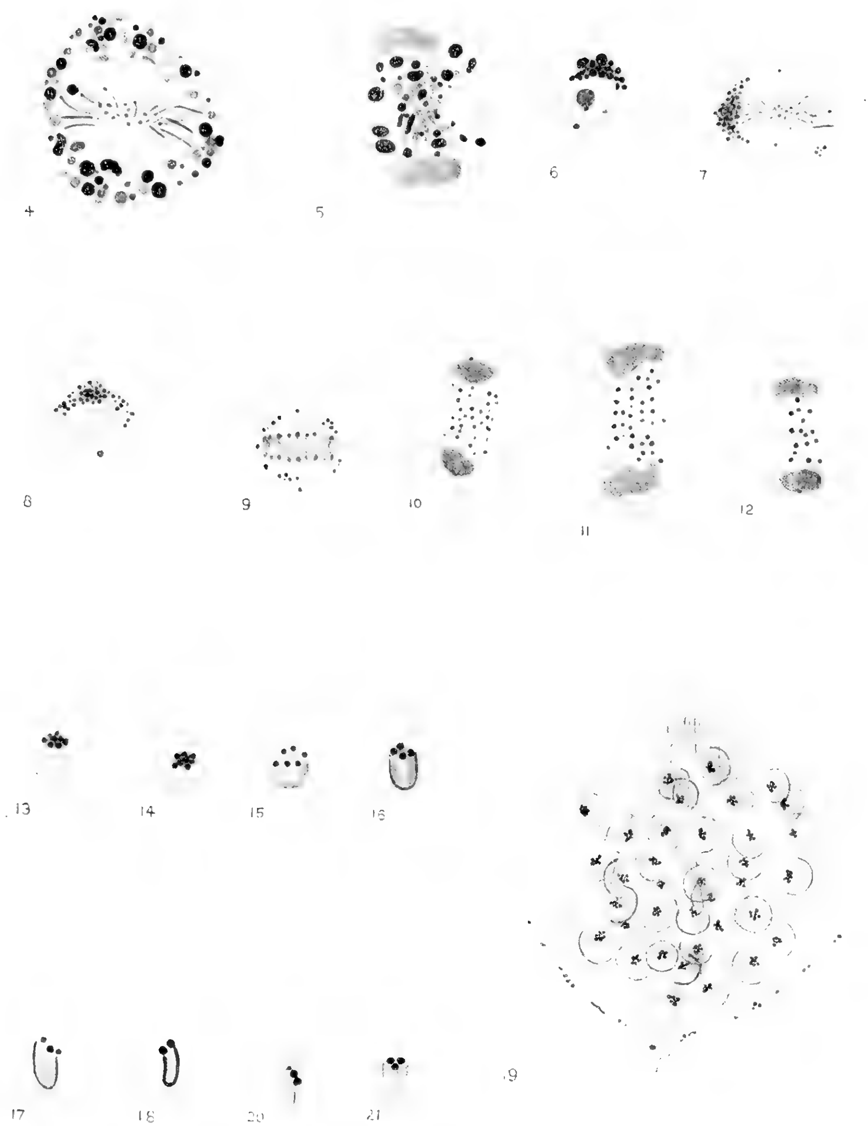
11 Fundulus heteroclitus. Fixation: Regaud. Stain: acid fuchsin-methyl-green. Anaphase of first division of maturation.

12 Same material. Anaphase of second division of maturation.

13 to 18 Same material. Six stages of spermiogenesis; in none is the tail represented in its full length. 16 and 17 are respectively face-view and side-view of approximatively the same stage.

19 Fundulus heteroclitus. Fixation: Benda. Stain: Benda. Group of spermatids in a cyst.

20 and 21 Fundulus majalis. Fixation: Benda, without acetic acid. Stain: Benda. Spermatozoa from the excretory ducts (the tail is not represented in its full length). 20: side-view; 21: face-view.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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE POSITION OF THE INSERTION OF THE PECTORALIS MAJOR AND DELTOID MUSCLES ON THE HUMERUS OF MAN

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THREE FIGURES

The metrical determination of the position of the attachments of muscles to bones is a problem which affords a contribution to topographical anatomy. It is also of importance from the standpoint of musculo-mechanics, because measuring the insertions of muscles is analogous to the determination of the lengths of leverage of the body. Since such an investigation promises to give information regarding differences or equalities of race or sex, as well as of the two halves of the body, it is of no less interest to the anthropologist. As far as the author can determine from a study of anatomical and anthropological literature, no measurements of the insertions of muscles have as yet been undertaken. In approaching this problem one is working in a new field of osteometry, where it becomes necessary to treat the bone not separately but in conjunction with the associated muscles, which have been so neglected in anthropometry.

The following study deals with the insertion of the pectoralis major and deltoid muscles. The measurements were made on the right and left arms of one hundred and five bodies. Forty-six of these bodies were obtained from the University of Maryland in Baltimore, forty from the Jefferson Medical College in Philadelphia and nineteen from the Johns Hopkins Medical School in Baltimore. The author wishes here to express his appreciation to Drs. W. H. Lewis, J. P. Schaeffer, J. Holmes Smith and J. W. Holland for their kindness in permitting the use of this material. All of the subjects measured were adults; juvenile and senile ones were excluded. It is regrettable that the sexes

were very unequally represented, for the females numbered only twenty-seven, as against seventy-eight male subjects. A greater uniformity occurred in race, as there were fifty-one white and fifty-four colored bodies. The author wishes to call attention to the fact that the term race is used in its widest sense in the present paper, because both the white and colored inhabitants of America have originated from numerous races in a limited sense. In negroes one frequently witnesses a more or less extensive admixture of white blood; in cases where there was evidence of a too great intermingling with the white element the material was discarded.

The position of the muscle insertion was compared with the length of the humerus by measuring the distance of the most proximal and the most distal point of attachment from the proximal end of the bone, and further by determining the arithmetical mean of these distances in percentage of the length of the humerus. For this purpose we need first of all six exact points of measurement, a proximal and a distal point on the humerus, two corresponding points on the pectoralis major and two more on the deltoid. The two points on the humerus are found by measuring the length of the bone, choosing the distance of the highest point of the caput humeri from the lowest point of the capitulum and measuring parallel to the axis of the bone (fig. 1, points I and II). The points of measurement for the pectoralis major muscle are the most proximal and the most distal points of its insertion on the crista tuberculi majoris (fig. 1, points III and IV); as a rule they are readily determined. Occasionally the distal portion of the insertion is intimately connected with the tendon of the deltoid muscle and the distal point can only be obtained after careful separation of these structures. In a limited number of cases the dorsal reflected portion of the muscle was observed to form a narrow tendinous band in the region where it spreads out proximally to join the tendinous lining of the sulcus intertubercularis (in figure 1 such an instance is indicated at *a*). In such cases this prolongation was ignored and the point of measurement taken at its distal end. The lower point of measurement of the deltoid is comparatively easy to

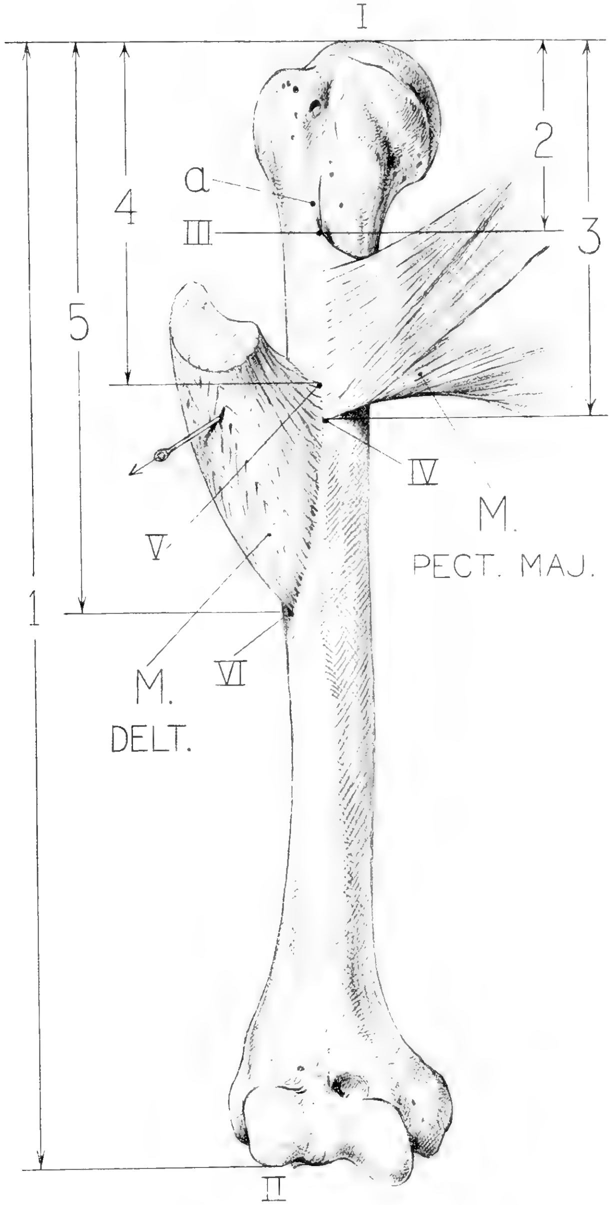

Fig. 1 Diagram of the points of measurement and distances on a right humerus seen from in front.

ascertain, namely as the most distal point of the insertion on the tuberositas deltoidea (fig. 1, point VI). The most proximal point of insertion is frequently concealed by the body of the muscle and it is necessary therefore to remove it partially. In doing this great care should be exercised as the deltoid is usually attached at its uppermost end by very delicate strands (fig. 1, point V). The distance between each of these four points and the highest point of the head of the humerus was measured parallel to the axis of the bone, similar to the longitudinal measurement of the humerous mentioned above and therefore these measurements are projections.

The measuring instrument employed was a modified small anthropometer of Martin (Stangenzirkel). This instrument is composed of a ruled metal bar or beam, possessing two arms at right angles to it, one of which is firmly attached to the end, the other movable in the direction of the bar, while both are movable at right angles to the latter. The modification consists merely in the addition of a third arm from another instrument of the same kind, which can also be moved both in the same direction and at right angles to the main axis (fig. 2).

First one measures the length of the humerus with the two outermost arms of the instrument holding the bar parallel to the axis of the bone, then the middle arm is approximated in turn to the four points of muscle insertion as defined above. This is performed by moving the arm up and down as required, shortening or lengthening it, simultaneously rotating the entire instrument around the axis of the humerus if necessary. Readings are taken each time on the ruled bar and correspond with the measurements two, three, four and five in figure 1. Indices for the relative position of the middle point of each muscle insertion were obtained by the following formulae:

$$\frac{\dfrac{\text{measurement } 2 + \text{measurement } 3}{2}}{\text{measurement } 1} \times 100 \text{ for the pectoralis major}$$

$$\frac{\dfrac{\text{measurement } 4 + \text{measurement } 5}{2}}{\text{measurement } 1} \times 100 \text{ for the deltoid}$$

The greater these indices of position, the more distal, the smaller, the more proximal is the insertion of the muscle. Following is a short description of the mathematical treatment of the length of the humerus and the indices which have been used in this paper. A more detailed explanation of these methods, which are absolutely necessary for an understanding of the

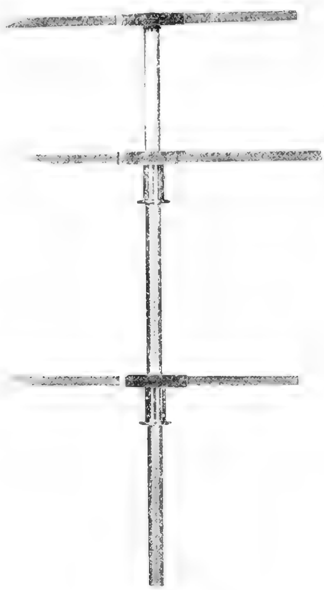

Fig. 2 Small anthropometer with three parallel movable arms.

measurements on a large number of individuals, is to be found in the Textbook of Anthropology by R. Martin, Jena, 1914, pp. 63–103.

The average (M) is the arithmetical mean of the individual values (V) of a group (n = number of individuals): $M = \frac{1}{n} \Sigma V$. The standard deviation (σ) is the square root of the average of

the squares of the deviations of the individual values from the average of the row and expresses the absolute variability: $\sigma = \sqrt{\frac{1}{n}\Sigma (V - M)^2}$. The variation coefficient (v) expresses the standard deviation in percentage of the average, whereby a criterion for the relative variability is obtained: $v = \frac{100\sigma}{M}$. The correlation coefficient (r) affords a means of determining the law, according to which two characteristics combine. It is the sum of the products of the deviations of the two characteristics from the corresponding averages taken for each individual, divided by the product of the number of individuals and the two standard deviations: $r = \frac{\Sigma (x - X)\ (y - Y)}{n\sigma_x \qquad \sigma_y}$. A complete correlation exists when $r = 1$. If $r = 0$, no relation prevails between the two characteristics. A positive correlation coefficient indicates a change of the characteristics in the same direction, a negative one, in the opposite direction. Finally, to test the degree of exactness of the above formulae, the probable error (E) was determined by the following formulae:

$$E\,(M) = \pm 0.6745 \frac{\sigma}{\sqrt{n}} \text{ for the average.}$$

$$E\,(\sigma) = \pm 0.6745 \frac{\sigma}{\sqrt{2n}} \text{ for the standard deviation.}$$

$$E\,(v) = \pm 0.6745 \frac{v}{\sqrt{2n}} \text{ for the variation coefficient.}$$

If $v > 10$, the last formula must be multiplied by $\sqrt{1 + 2\left(\frac{v}{100}\right)^2}$

$$E\,(r) = \pm 0.6745 \frac{1 - r^2}{\sqrt{n}} \text{ for the correlation coefficient}$$

The relation of the insertion of the muscles to the length of the humerus makes a short preliminary discussion of this absolute measurement necessary. Table 1 is a compilation of the averages and the conditions of variability of the length of the two hundred and ten humeri, which were measured. The extremes of these measurements range from 260 to 367 mm. The humerus in male whites is on the average 26 mm., in male negroes 31.8 mm.

TABLE 1

Averages, standard deviations, variation coefficients, their probable errors and ranges of variation for the length of the humerus

RACE	SEX	NUMBER	SIDE	$M \pm E(M)$	$\sigma \pm E(\sigma)$	$v \pm E(v)$	Minimum	Maximum
Whites	♂	40	r.	316.3±1.71	15.94±1.22	5.04±0.38	283	347
	♂	40	l.	316.5±1.82	17.05±1.29	5.40±0.41	283	352
	♂	80	r. l.	316.4±1.25	16.50±0.87	5.22±0.27	283	352
	♀	11	r.	293.1±2.58	12.65±1.81	4.32±0.62	269	309
	♀	11	l.	287.7±2.02	9.92±1.42	3.44±0.50	267	303
	♀	22	r. l.	290.4±1.67	11.69±1.19	4.03±0.41	267	309
Negroes	♂	38	r.	326.2±1.87	17.18±1.34	5.27±0.41	290	367
	♂	38	l.	323.5±2.02	18.50±1.44	5.71±0.44	283	365
	♂	76	r. l.	324.8±1.39	17.89±0.98	5.50±0.30	283	367
	♀	16	r.	294.6±2.45	14.51±1.74	4.92±0.59	266	321
	♀	16	l.	291.5±2.51	14.84±1.78	5.10±0.61	260	312
	♀	32	r. l.	293.0±1.77	14.75±1.24	5.03±0.42	260	321

longer than in females. The averages in negroes exceed in both sexes the corresponding values for whites. The division of table 1 into separate rows for the right and left humerus shows that the variability is greater on the left side except in the group of white females of which the number measured was quite small. Furthermore it shows that the white males, who possess the same average length of the humerus on both sides, form an exception to the rule of the greater length of the humerus on the right side. Table 2, which gives a survey of the absolute and

TABLE 2

Absolute and relative numbers of individuals with equal and different lengths of the humeri and average differences of the individual asymmetries (mm.)

RACE	SEX	BOTH SIDES EQUAL	RIGHT SIDE LONGER	LEFT SIDE LONGER	AVERAGE DIFFERENCE IF	
					Right side longer	Left side longer
Whites	♂	10=25.0%	17=42.5%	13=32.5%	3.88	5.62
	♀	0= 0.0%	9=81.8%	2=18.2%	7.67	5.00
Negroes	♂	8=21.0%	22=58.0%	8=21.0%	5.73	3.25
	♀	7=43.8%	9=56.2%	0= 0.0%	5.44	0

relative number of cases possessing humeri of equal and different lengths and the average differences of the individual asymmetries, shows that 32.5 per cent of white males have a longer left humerus. It also demonstrates that in white males the differences in favor of the left side are on the average greater than those on the right, which is not the case in the other groups. The greatest absolute asymmetry occurred in a negro whose right humerus exceeded the left in length by 23 mm.

TABLE 3

Averages, standard deviations, variation-coefficients, their probable errors and ranges of variation for the position index of the insertion of the pectoralis major muscle

RACE	SEX	NUMBER	SIDE	$M \pm E\ (M)$	$\sigma \pm E\ (\sigma)$	$v \pm E\ (v)$	MINIMUM	MAXIMUM
Whites	♂	40	r.	28.37±0.17	1.55±0.12	5.46±0.41	24.3	31.3
	♂	40	l.	28.50±0.16	1.49±0.11	5.23±0.40	25.3	31.8
	♂	80	r. l.	28.43±0.11	1.52±0.08	5.35±0.28	24.3	31.8
	♀	11	r.	26.37±0.35	1.74±0 25	6.59±0.94	21.9	28.3
	♀	11	l.	26.35±0.56	2.73±0.39	10.34±1.48	21.3	29.5
	♀	22	r. l.	26.36±0.33	2.29±0.23	8.67±0.88	21.3	29.5
Negroes	♂	38	r.	28 27±0.19	1.75±0.14	6.18±0.48	26.1	35.2
	♂	38	l.	27.99±0.17	1.57±0.12	5.61±0.44	25.1	32.6
	♂	76	r. l.	28.13±0.13	1 67±0.09	5.94±0.33	25.1	35.2
	♀	16	r.	26.82±0.32	1.89±0 23	7.05±0.85	23.1	30.9
	♀	16	l.	26.45±0.31	1.82±0 22	6 89±0.83	22.3	30.3
	♀	32	r. l.	26.63±0.22	1.86±0.16	6.99±0.59	22.3	30.9

The averages and the conditions of variability of the index of position for the middle of the insertion of the pectoralis major muscle are given in table 3. This index differs in the entire material between 21.3 and 35.2. Expressing this in terms of the mechanics of levers, one can state that in the adult the lifting arm of the musculus pectoralis major is related to the carrying arm—the length of the humerus—in a ratio varying from 21.3: 100 to 35.2: 100. In other words the relation of the lever arms may differ by almost 14 per cent of the length of the carrying arm, and this expressed in an absolute number equals on the average about 45 mm. A different proportion of the lever arms influences not only the force of the muscle but also the movement

of the lever, if the shortening of the muscles is equal. This can be readily seen from the diagram (fig. 3) $A - B$ represents the humerus, its caput at A. C and D correspond to the two most extreme points of insertion of the pectoralis major. 1 and 2 indicate the two appertaining muscles. Should the latter shorten by the same amounts $C - C' = D' - D'$, than the lower end of the humerus B is moved more extensively by muscle 1 (to B'') than by muscle 2 (to B'), for instance the humerus is turned through a greater angle when the muscles are contracting equally by the more proximal one, and consequently also more quickly. A more distally situated pectoralis major would have to contract more in order to pull the arm forward to a certain

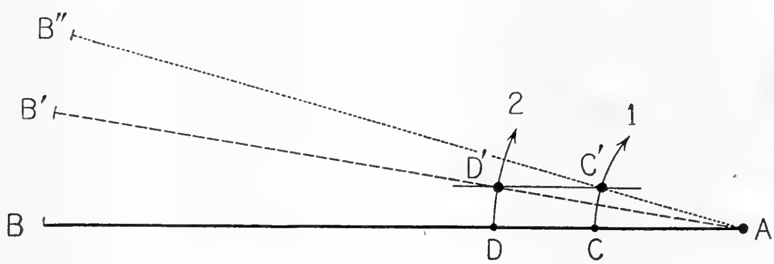

Fig. 3 Diagram of the movements of the humerus with equal shortening of the pectoralis major muscle at different points of attachment.

angle than would be necessary if it were more proximally attached. With increased contraction, however, a muscle loses in tension and consequently the greater shortening of the more distal muscle diminishes the advantage of its favorable lever arm.

In the group of whites as well as in the negroes, the averages of the index of position in the males exceed those of the females, the differences being 2.07 and 1.50 respectively. Since the probable errors of these averages are only small, this sexual difference, such as that the female possesses a more proximally attached pectoralis major muscle, must be considered as a rather essential and definite one. It is not only of interest in connection with the above mentioned consideration of musculo-mechanisics, but also

indicates that the female arm is looser, that is the perpendicular diameter of the axilla in the female is relatively shorter since the caudal edge of the pectoralis major at its lateral end represents the lower border of the axilla. The average of both sexes in the whites hardly differs from that found in the negroes; the two races are alike therefore in regard to the position of the insertion of the pectoralis major. The variation coefficient of the index is always rather high and even exceeds 10 in one case. The conclusion can be drawn from this that the position of the attachment of the pectoralis major can be only slightly influenced by the length of the humerus, since the index connecting these two

TABLE 4

Absolute and relative numbers of individuals with symmetrical and asymmetrical position of the pectoralis major insertion and averages of the individual differences of the position index

RACE	SEX	THE SAME POSITION ON BOTH SIDES	POSITION ON THE RIGHT MORE DISTAL	POSITION ON THE LEFT MORE DISTAL	AVERAGE DIFFERENCE IF MORE DISTALLY	
					on the right	on the left
Whites	♂	1=2.5%	17=42.5%	22=55.0%	1.38	1.29
	♀	0=0.0%	4=36.4%	7=63.6%	2.17	1.21
Negroes	♂	2=5.2%	18=47.4%	18=47.4%	1.70	1.10
	♀	0=0.0%	13=81.2%	3=18.8%	0.62	0.73

is quite variable. In order to throw some light on the question concerning the relationship of the point of attachment and the strength of the muscle, the individuals of each group were divided into four subgroups, namely into weak, medium, strong and very strong ones. Positive and negative variants of the index of position were found to be indiscriminately distributed among the four subgroups, in whites as well as in negroes and in males as well as in females. The strength of the muscle has therefore no influence on the position of the attachment. Table 4 shows a grouping of the absolute and relative number of individuals with symmetrical and asymmetrical position of muscle attachment, and the averages of the individual differences in the index of position. The position of attachment of

the pectoralis major muscle was the same on both sides in only three out of one hundred and five individuals. The unusually large percentage of asymmetrical cases is on the average equally distributed on the two sides. One finds only small differences between the averages on the right and left side in table 3. The largest individual asymmetry was found in a negro whose index of position was 6.0 greater on the right than on the left side.

The index which has just been discussed gives a clear idea of the position of the median point of the muscle insertion, and it will therefore be of interest to devote our attention briefly to the length of the insertion of the pectoralis major, from which the median point was obtained. The absolute value of this insertion length is represented by the difference between measurement 2 and 3 in figure 1. In order to make this measurement independent of the individual size of the upper arm it has been expressed in percents of the humerus length. The formula of this relative measurement is as follows:

$$\frac{\text{measurement 3} - \text{measurement 2}}{\text{measurement 1}} \times 100$$

The averages and the conditions of variability of this index are tabulated in table 5. One notices a tremendous range of variation from 8.8 to 23.1, and the variation coefficients also are unusually large. It seems inadvisable therefore to attach any particular significance to the slight differences in sex and race, such as the relatively longer attachment of the muscle in females and in whites. There is no correlation between the relative insertion length and the muscle strength nor the position of the insertion. The measurement which has just been discussed is somewhat longer in whites on the right side, and in negroes on the left. There is a very marked tendency to asymmetry in the relative insertion lengths in the different individuals, as has already been found to be the case for the position of the insertion. The relative attachment length was equal on both sides in only four cases, and in only one case did the absolute length of attachment show no asymmetry.

TABLE 5

Averages, standard deviations, variations-coefficients, their probable errors and ranges of variation for the relative length of the insertion of the pectoralis major muscle

RACE	SEX	NUMBER	SIDE	$M \pm E\ (M)$	$\sigma \pm E\ (\sigma)$	$v \pm E\ (v)$	MINIMUM	MAXIMUM
Whites	♂	40	r.	16.84±0.25	2.37±0.18	14.11±1.09	9.2	23.1
	♂	40	l.	16.32±0.25	2.35±0.18	14.42±1.12	11.5	20.8
	♂	80	r. l.	16.58±0.18	2.36±0.12	14.22±0.77	9.2	23.1
	♀	11	r.	16.95±0.39	1.94±0.28	11.48±1.66	13.6	20.3
	♀	11	l.	16.53±0.50	2.45±0.35	14.85±2.16	12.9	21.5
	♀	22	r. l.	16.74±0.32	2.22±0.23	13.29±1.38	12.9	21.5
Negroes	♂	38	r.	16.13±0.38	3.52±0.27	21.86±1.77	8.8	22.7
	♂	38	l.	16.30±0.24	2.18±0.17	13.37±1.06	10.5	20.2
	♂	76	r. l.	16.21±0.23	3.02±0.16	18.64±1.02	8.8	22.7
	♀	16	r.	15.90±0.41	2.42±0.29	15.22±1.88	12.3	21.9
	♀	16	l.	16.88±0.38	2.27±0.27	13.43±1.64	12.0	20.4
	♀	32	r. l.	16.39±0.28	2.36±0.20	14.39±1.23	12.0	21.9

The averages and the conditions of variation of the index, which expresses the relative position of the middle point of the insertion of the deltoid muscle, are given in table 6. The variation extends from 34.0 – 46.5, that is it equals 31 per cent of the middle value of the two extremes and therefore remains

TABLE 6

Averages, standard deviations, variation-coefficients, their probable errors and ranges of variation for the position index of the insertion of the deltoid muscle

RACE	SEX	NUMBER	SIDE	$M \pm E\ (M)$	$\sigma \pm E\ (\sigma)$	$v \pm E\ (v)$	MINIMUM	MAXIMUM
Whites	♂	40	r.	40.45±0.22	2.03±0.15	5.02±0.38	37.2	44.8
	♂	40	l.	41.17±0.19	1.80±0.14	4.37±0.33	37.5	45.6
	♂	80	r. l.	40.81±0.15	1.94±0.10	4.76±0.25	37.2	45.6
	♀	11	r.	39.35±0.52	2.57±0.37	6.52±0.93	34.0	42.5
	♀	11	l.	40.61±0.42	2.06±0.29	5.07±0.72	36.4	42.9
	♀	22	r. l.	39.98±0.34	2.41±0.24	6.03±0.61	34.0	42.9
Negroes	♂	38	r.	40.40±0.24	2.22±0.17	5.50±0.43	34.0	44.7
	♂	38	l.	40.52±0.22	2.00±0.16	4.94±0.38	36.2	44.6
	♂	76	r. l.	40.46±0.16	2.12±0.12	5.24±0.29	34.0	44.7
	♀	16	r.	40.64±0.48	2.86±0.34	7.04±0.84	34.3	46.5
	♀	16	l.	41.11±0.42	2.50±0.30	6.08±0.73	36.1	45.0
	♀	32	r. l.	40.87±0.32	2.70±0.23	6.60±0.55	34.3	46.5

considerably less than the variability of the position index of the pectoralis major, the variation of which equaled 49 per cent of its mean. The last named index shows in every group a greater variation coefficient than the corresponding ones in table 6. Therefore the deltoid possesses a more constant position of insertion than the pectoralis major muscle. Judging from the averages of the position index the attachment of the deltoid muscle must be relatively slightly more distal in the males of the white race, and slightly more proximal in the males of the negroes than in the females of either. There is no difference in the two races in the position of the insertion of the deltoid, similar to that found to be the case for the pectoralis major. The relative position of the deltoid insertion is also almost regularly unequal on both sides; more frequently the muscle is more proximally situated on the right side. In all the groups the averages of the position index of the deltoid are on the right—in part even considerably—smaller than those of the left side. A relationship between the strength of the deltoid muscle and its insertion position does not exist. The question as to what extent the positions of the insertions of the pectoralis major and the deltoid may change correspondingly is best answered by the following tabulation of the correlation coefficients with their probable errors for the two indices of position which have been previously used. White males $+ 0.52 \pm 0.057$, white females $+ 0.37 \pm 0.123$, negro males $+ 0.29 \pm 0.071$, negro females $+ 0.70 \pm 0.061$. The coefficients, which are regularly positive, indicate that a shifting of one of the muscles is usually followed by a change to a greater or less extent of position of the other muscle in the same direction. This is very noticeable in female negroes and in male whites. In the material used the proximal point of measurement of the insertion of the deltoid was found above the distal point of measurement of the pectoralis major muscle insertion in one hundred and eighty-six cases; sixteen times the points referred to were at the same height and in only eight cases was the first point found below the latter.

The most proximal region of insertion of the deltoid is much more variable than the most distal. In order to free the index

of position, which has just been discussed and which uses the mean value depending on the two terminal points of the insertion, from the great variability of the upper point it was found necessary to calculate a second position index for the deltoid, employing only the most distal point of insertion. This new index gives information as to how far down the deltoid extends upon the humerus. The formula for this index, using the measurements of figure 1, reads as follows:

$$\frac{\text{measurement } 5}{\text{measurement } 1} \times 100$$

The averages and conditions of variability of the index of the relative position of the most distal point of attachment of the deltoid are given in table 7. The entire variation reaching from 44.8 to 57.5 comprises 25 per cent of the mean obtained from the end values just cited; it is therefore relatively much smaller than the variation of the preceding index, which employed the middle point of insertion. The variation coefficients in table 7 lie in all the groups below those in table 6 and should be considered as relatively small. The position of the most distal point

TABLE 7

Averages, standard deviations, variation-coefficients, their probable errors and ranges of variation for the position index of the most distal point of insertion of the deltoid muscle

RACE	SEX	NUMBER	SIDE	$M \pm E(M)$	$\sigma \pm E(\sigma)$	$v \pm E(v)$	MINIMUM	MAXIMUM
Whites	♂	40	r.	50.13±0.21	1.98±0.15	3.95±0.30	44.8	55.0
	♂	40	l.	50.81±0.21	1.96±0.15	3.86±0.29	47.9	57.5
	♂	80	r. l.	50.47±0.15	1.97±0.10	3.90±0.21	44.8	57.5
	♀	11	r.	49.88±0.35	1.74±0.25	3.49±0.50	45.6	52.5
	♀	11	l.	50.34±0.36	1.76±0.25	3.50±0.50	47.3	53.9
	♀	22	r. l.	50.11±0.25	1.76±0.18	3.51±0.36	45.6	53.9
Negroes	♂	38	r.	49.59±0.16	1.50±0.12	3.02±0.23	46.2	52.8
	♂	38	l.	50.18±0.15	1.41±0.11	2.81±0.22	46.5	52.9
	♂	76	r. l.	49.88±0.12	1.48±0.08	2.97±0.16	46.2	52.9
	♀	16	r.	48.96±0.39	2.31±0.28	4.71±0.56	45.1	54.8
	♀	16	l.	49.52±0.37	2.21±0.27	4.46±0.53	45.0	54.1
	♀	32	r. l.	49.24±0.27	2.27±0.19	4.61±0.39	45.0	54.8

of the deltoid is accordingly the most constant of the points of muscle insertion which have been used, and can be located with considerable precision near the middle point of the humerus. Actually, this point lies slightly below the middle of the length of the humerus in whites, slightly above in negroes; a racial difference which is represented by the average difference of 0.73 of the averages of the index. In the female the most distal point of the deltoid is situated somewhat more proximal than in the male. It is also of interest to note, that the distal end point of the deltoid insertion, similarly to the middle point, is located on the average noticeably more distally on the left side than on the

TABLE 8

Absolute and relative numbers of individuals with symmetrical and asymmetrical position of the most distal point of insertion of the deltoid and averages of the individual differences of the position index

RACE	SEX	THE SAME POSITION ON BOTH SIDES	POSITION ON THE RIGHT MORE DISTAL	POSITION ON THE LEFT MORE DISTAL	AVERAGE DIFFERENCE IF MORE DISTALLY	
					on the right	on the left
Whites	♂	0=0.0%	13=32.5%	27=67.5%	1.28	1.61
	♀	1=9.1%	5=45.4%	5=45.4%	1.28	2.28
Negroes	♂	2=5.2%	12=31.6%	24=63.2%	1.08	1.47
	♀	0=0.0%	6=37.5%	10=62.5%	0.73	1.34

right. Table 8 gives a view of the absolute and relative number of individuals with equal and unequal indices of position for the most distal point of the deltoid insertion and also the average differences of the unequal indices. It shows that the point referred to occupies the same relative position on both sides in a total of only three cases; furthermore that the point was in a greater number of instances more distally situated on the left side, and that the differences in favor of the left always exceeded on the average those of the right. The greatest individual difference of the index appears in a white male with 6.4 in favor of the left side.

The exact determination of the position of the insertion of the pectoralis major and deltoid muscles on the humerus shows,

when briefly summarized, the possibilities of variation, the more constant position of the deltoid insertion compared with the pectoralis major, the equality of the positions of the two muscle insertions in whites and negroes, the relatively higher attachment of the pectoralis major in females, and lastly the surprisingly common asymmetry of the insertion positions. The author has noticed that asymmetries of position of the insertions occurred as early as birth, although they are not as frequent nor as marked in the newborn as in adults.

In conclusion, the individual relative measurements and humerus lengths which form the basis of this paper are tabulated, and are arranged according to increasing lengths of the right humerus.

White males

HUMERUS LENGTH		POSITION INDEX FOR THE INSERTION OF THE PECTORALIS MAJOR		RELATIVE LENGTH OF THE INSERTION OF THE PECTORALIS MAJOR		POSITION INDEX FOR THE INSERTION OF THE DELTOID		POSITION INDEX FOR THE MOST DISTAL POINT OF INSERTION OF THE DELTOID	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
283	283	31.3	31.8	16.6	19.8	41.7	41.9	49.8	50.9
286	286	28.5	27.8	13.6	11.5	42.3	41.4	52.4	51.7
289	294	28.4	29.6	15.2	12.2	44.8	43.0	55.0	53.1
296	303	27.2	28.5	20.6	14.2	42.6	41.7	47.6	47.9
299	291	27.9	28.4	18.4	15.5	42.3	40.7	50.2	52.2
299	298	29.9	30.2	23.1	20.8	41.8	41.8	50.5	51.0
299	301	27.4	29.2	17.4	16.6	37.6	42.0	47.2	50.2
300	300	29.0	26.0	16.7	18.7	39.3	39.0	48.3	50.0
302	294	29.0	26.0	12.3	13.9	42.9	42.7	50.7	51.4
302	296	28.5	26.9	15.9	15.9	42.7	41.0	51.3	48.3
302	311	29.5	30.2	15.9	12.2	41.1	42.1	49.7	51.4
304	303	25.0	27.9	18.4	17.5	37.3	43.1	48.4	50.8
306	313	27.8	28.9	15.7	16.0	37.4	39.3	49.6	49.8
307	307	28.7	28.7	20.8	15.6	36.5	39.7	50.5	51.5
311	310	29.7	27.0	17.0	16.5	40.4	40.3	50.2	48.7
314	312	30.1	29.8	9.2	17.3	38.5	39.6	51.3	52.6
315	311	26.8	27.5	15.0	12.5	40.3	39.2	49.8	48.9
315	315	27.5	30.6	17.5	20.0	43.0	45.6	51.1	57.5
315	315	26.7	27.5	19.0	18.7	39.7	41.6	51.1	50.2
317	310	28.2	27.7	19.2	16.8	38.6	43.2	51.7	51.6
319	318	27.0	28.9	16.9	14.5	40.6	41.2	50.8	50.3
319	319	28.1	30.3	17.9	17.2	40.4	43.9	50.2	52.0
320	315	30.2	31.1	16.6	14.0	43.1	43.3	53.7	52.4
321	320	25.5	25.3	19.3	20.0	38.3	37.5	47.7	48.4
321	326	28.8	30.5	14.6	16.9	39.6	41.9	52.6	54.0
323	318	27.9	27.5	18.0	16.0	40.6	39.6	49.8	48.1
323	327	24.3	27.1	17.0	19.3	38.5	40.4	47.7	48.3
324	320	29.6	29.4	19.1	16.9	42.1	39.5	53.1	51.3
328	329	30.5	30.1	15.2	18.5	42.7	43.0	50.9	52.3
329	327	28.9	27.7	15.2	16.2	40.3	40.7	48.9	50.5
332	343	27.1	27.7	15.7	16.9	38.1	40.1	46.4	48.1
333	333	30.0	27.2	16.8	13.5	39.6	40.2	50.5	51.4
334	327	29.0	28.9	14.4	13.8	40.9	41.3	49.1	48.9
334	336	29.6	28.6	17.4	19.0	40.4	42.1	51.2	51.8
334	336	29.9	30.8	19.2	17.0	43.0	45.2	51.5	54.2
334	346	31.1	27.5	15.6	15.6	43.6	39.6	51.8	49.7
335	332	29.1	27.4	15.8	18.7	38.9	39.8	44.8	49.1
335	335	26.9	27.6	16.1	18.2	39.3	39.1	49.3	49.6
346	352	27.2	28.6	17.9	13.9	39.9	41.9	51.2	52.6
347	347	27.1	27.5	17.3	14.7	37.2	37.8	47.8	49.6

White females

HUMERUS LENGTH		POSITION INDEX FOR THE INSERTION OF THE PECTORALIS MAJOR		RELATIVE LENGTH OF THE INSERTION OF THE PECTORALIS MAJOR		POSITION INDEX FOR THE INSERTION OF THE DELTOID		POSITION INDEX FOR THE MOST DISTAL POINT OF INSERTION OF THE DELTOID	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
269	267	26.2	28.8	18.2	15.7	42.2	42.9	49.8	49.4
279	278	26.9	28.5	13.6	16.5	36.2	41.7	49.1	50.4
281	280	27.0	27.7	17.1	14.6	37.7	37.7	49.5	49.6
284	290	28.3	25.9	15.8	17.2	42.3	41.7	51.4	51.4
290	281	24.1	21.5	14.5	17.4	38.1	38.8	50.0	47.3
299	288	27.8	29.5	18.1	21.5	42.5	42.2	51.5	51.4
299	303	21.9	21.3	17.1	19.5	39.6	39.9	48.8	49.2
301	298	27.1	27.3	20.3	17.8	40.5	41.8	52.5	52.0
306	290	27.1	24.0	19.6	15.5	39.2	36.4	51.0	48.3
307	295	26.5	27.5	16.0	12.9	34.0	40.7	45.6	50.8
309	295	27.2	27.9	16.2	13.2	40.6	42.9	49.5	53.9

Negro males

290	283	26.6	26.3	17.2	18.7	41.4	42.0	48.3	48.4
298	298	29.2	31.0	14.8	15.8	40.0	42.1	47.0	49.7
301	292	27.7	28.1	18.3	17.1	40.5	41.4	48.5	49.7
303	301	29.5	26.9	14.2	12.6	40.3	40.7	49.8	49.8
307	310	27.9	28.0	16.6	17.1	38.6	36.6	49.8	50.0
308	303	27.9	28.5	16.2	16.8	42.2	42.7	51.0	50.8
308	307	26.5	25.7	18.5	20.2	37.0	37.1	49.7	51.1
312	306	27.9	28.9	17.3	15.4	39.1	40.5	50.0	50.0
312	307	30.6	32.6	9.3	17.6	43.9	41.9	50.0	51.1
313	313	29.6	29.6	22.7	17.6	42.5	41.7	51.4	49.5
318	316	27.7	28.5	13.8	18.4	38.2	41.9	51.3	51.0
320	314	28.9	27.2	9.1	16.9	41.4	40.4	51.0	50.6
320	319	27.3	25.1	21.6	19.4	39.8	37.1	50.6	51.1
322	316	26.1	25.8	18.0	15.5	43.3	42.9	51.6	52.9
322	316	26.9	26.9	14.6	13.9	40.8	42.1	49.7	50.3
322	322	26.6	29.0	18.9	15.2	38.8	41.9	50.0	48.5
322	322	28.9	29.5	14.3	19.9	40.5	41.6	48.1	49.7
323	316	27.2	26.4	21.7	15.5	35.6	36.2	49.5	48.4
323	318	28.9	30.0	19.5	17.3	40.7	42.5	48.9	51.6
323	323	29.7	29.3	16.7	14.5	41.8	41.6	48.3	50.8
325	328	26.3	29.0	8.9	11.0	34.0	37.0	46.2	50.9
328	305	29.7	28.5	8.8	10.5	39.3	39.5	48.5	51.5
328	327	29.3	27.7	19.5	18.0	44.7	40.5	49.7	49.8
329	329	28.7	28.0	16.7	14.0	40.6	39.1	49.2	46.5
330	330	27.6	27.4	14.5	16.7	37.3	40.6	50.0	51.8
331	324	28.1	26.7	15.7	13.3	38.7	37.2	48.3	48.8

Negro males—Continued

HUMERUS LENGTH		POSITION INDEX FOR THE INSERTION OF THE PECTORALIS MAJOR		RELATIVE LENGTH OF THE INSERTION OF THE PECTORALIS MAJOR		POSITION INDEX FOR THE INSERTION OF THE DELTOID		POSITION INDEX FOR THE MOST DISTAL POINT OF INSERTION TO THE DELTOID	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
332	333	26.5	26.9	16.9	15.3	40.2	40.7	49.4	51.7
337	332	35.2	29.2	11.6	15.7	40.8	40.2	48.4	48.8
338	339	30.5	26.8	20.7	17.7	40.2	37.9	48.8	47.8
339	347	29.1	30.3	20.9	17.9	43.8	42.7	52.8	51.0
342	342	27.2	28.2	16.4	16.7	43.9	44.6	51.8	52.6
342	344	29.5	27.2	11.7	14.8	39.3	39.8	47.1	48.3
345	352	28.0	29.1	14.8	17.3	41.6	41.2	48.4	48.6
349	350	28.7	26.3	16.0	17.7	39.7	40.9	49.0	50.0
350	348	26.9	27.0	16.0	19.0	42.4	41.7	51.1	49.7
355	352	24.4	25.9	17.2	17.0	40.4	38.8	47.9	49.7
360	345	27.8	28.7	14.4	15.1	42.1	40.3	52.8	52.2
367	365	29.3	27.4	18.8	16.4	39.8	42.1	50.4	52.1

Negro females

266	260	27.3	25.6	13.2	15.8	41.2	41.7	48.5	50.0
272	269	27.0	26.8	15.8	17.1	42.6	42.2	50.0	50.6
280	271	28.7	27.3	14.6	14.0	40.4	42.1	47.9	50.9
283	279	30.9	30.3	16.6	20.4	46.5	45.0	54.8	54.1
285	285	27.4	28.4	14.7	17.5	41.9	42.3	50.2	49.5
286	286	26.7	26.2	15.0	14.7	37.4	39.3	45.1	45.5
290	290	28.3	27.9	13.1	16.6	41.7	41.7	51.4	51.0
297	297	27.4	26.8	21.9	19.9	41.8	43.8	49.2	50.8
301	296	24.9	25.8	18.6	18.6	38.0	38.0	48.8	48.6
301	301	23.1	22.3	12.3	12.0	38.7	38.7	47.2	47.8
302	302	23.7	23.5	16.9	17.9	37.1	37.4	48.3	49.0
303	298	26.9	26.8	15.5	15.4	41.7	42.4	49.8	50.3
308	302	25.3	25.0	19.5	19.5	34.3	36.1	46.1	45.0
309	307	25.6	25.9	16.2	15.3	43.0	40.9	49.5	48.2
309	309	27.7	27.5	16.5	19.4	43.4	44.8	50.8	52.1
321	312	28.2	27.2	14.0	16.0	40.5	41.3	45.8	49.0

AUTHOR'S ABSTRACT OF THIS PAPER
ISSUED BY THE BIBLIOGRAPHIC SERVICE

THE EFFECT OF THE HEART-BEAT UPON THE DEVELOPMENT OF THE VASCULAR SYSTEM IN THE CHICK

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SEVENTEEN FIGURES

CONTENTS

I. INTRODUCTION

At the time the circulation begins in the chick, the embryo possesses a number of relatively large blood vessels. Thoma ('93) mentions that the paired dorsal aortae are present, and in a schematic drawing (p. 32) shows them connecting, anteriorly, through capillaries with the heart, and, posteriorly, extending into the capillary net of the extra-embryonic region.

Miss Sabin ('17) in a monograph published recently,states that, in addition to the dorsal aortae, the duct of Cuvier, the cardinals, and several neural vessels have formed. These are represented in plate 1, figures 2 and 3, and in plate 2, figures 1 and 2 of the monograph, which also show the primitive anterior vitelline veins. She describes the vessels of the extra-embryonic region as an extensive capillary plexus which connects with the heart and dorsal aortae of the embryo. Quoting in part, she

says: ". . . . there is a plexus of vessels covering the entire area opaca and area pellucida which connects with the venous end of the heart and with the entire dorsal aorta of the embryo opposite the zone of the myotomes." That the heart-beat has much to do with the further development of this primitive vascular system has been generally conceded, and Thoma has formulated his three well-known histo-mechanical laws, which, as translated into English by Bruce ('96, p. 266), are as follows: "The increase in size of the lumen of the vessel, or what is the same thing, the increase in surface of the vessel wall, depends upon the rate of the blood current." "The growth in thickness of the vessel wall is dependent upon its tension. Further, the tension of the wall is dependent upon the diameter of the lumen of the vessel and upon the blood-pressure." "Increase of the blood-pressure in the capillary areas leads to new formation of capillaries." In order to test the validity of this view, it was decided to remove the heart by surgical methods before the establishment of a circulation, thereby eliminating most of the mechanical factors mentioned by Thoma, and then to study the further development of the vascular system in the area vasculosa.

II. REVIEW OF LITERATURE

Roux ('78) briefly mentions seeing an area vasculosa of a five-day chick in which the embryo was missing. He describes a simple capillary plexus with a mesh-like arrangement, and without formation of arteries and veins. In a note added later ('95), he mentions seeing further cases of chick embryos in which the embryo had failed to develop, but in which the sinus teminalis had formed. He refers also, in a very indefinite way, to the presence in these embryos of large vessels which corresponded somewhat in position and in direction to the normal arteries and veins. He gives no illustrations.

Loeb ('93) tested the effect of solutions of potassium chloride on Fundulus eggs, and observed that the living embryos developed without a heart-beat, the heart being thrown into a state of tetanus by the potassium. In spite of this some vessels

were formed. This work was later confirmed, in a general way, by Stockard ('06), although he states that the vascular development was far from normal.

Knower ('07) studied the effects of the early removal of the heart and arrest of the circulation on the development of frog embryos. In this experiment the heart was removed by cutting. Some of the embryos lived and continued to develop for as long as fourteen days. Many of the main blood vessels developed, although they were irregularly distended and abnormal.

Patterson ('09) studied the development of the extra-embryonic vascular system in chicks in which the embryo had been prevented from forming by making injuries on the unincubated blastoderm. He states (pp. 87–88), "although there is not the slightest trace of the embryo proper present, yet the vascular system of the area opaca is well laid down, and even large vessels are seen to pass inwards to the center of the pellucid area." This is illustrated in figure 7, p. 89, of his article.

Stockard ('15) made extensive studies, using chemical methods somewhat similar to J. Loeb's on Fundulus embryos. He found that in embryos in which there had been no heart-beat, the aorta developed into a vessel of considerable size, and he mentions that other vessels had also developed. He pictures only a cross-section of the aorta, however, and his reference to the other vessels is very meager.

It seems clear, then, from the observations of Thoma, and Sabin, that before the circulation of blood commences, a considerable development of the vascular system has taken place. This includes, in the embryo proper, the development of definite aortae, of short stretches of the two vitelline veins, of some of the dorsal segmental arteries, of part of the cardinal veins, and certain neural vessels in the head region, while, in the extra-embryonic area, there is present an indifferent net-work of capillaries, to which we might add (Lillie, '08, pp. 87–88) the sinus terminalis. This much obviously develops as the result of hereditary influences. Soon after the circulation starts, certain other arteries and veins begin to be marked out, and extensive further changes take place within a few days.

The question now arises, whether this further development of the vascular system, which takes place after the circulation is established is dependent upon the mechanical factors concerned with the circulation or not. According to Thoma, it is, while the observations of Roux and of Patterson indicate that the sinus terminalis will develop in the absence of a heart-beat, and that possibly the same may be true of other yolk-sac vessels. The observations of Loeb, Knower, and Stockard indicate the development of some main vessels when no circulation is present, but do not give a complete picture. It therefore still remains to be determined, precisely, how much vascular development takes place independently of the circulation.

III. METHOD OF INVESTIGATION

The chick was selected for this study on account of its rapid development, because it is possible to secure material for experimentation during most of the year, and because of the ease of injecting and studying the extra-embryonic vessels, which lie in a single plane, and which early develop a very characteristic pattern. After some experimentation, I decided upon the thirty-third hour of incubation as the proper time for operation, and afterwards, endeavored to arrange my operations so that they would come as nearly the hour mentioned as possible. At this time, the chick has about twelve somites. Miss Sabin has observed that the heart begins beating at the ten somite stage, but that the circulation does not start until after fifteen or sixteen somites have formed, which would mean about the thirty-eighth hour of incubation. Therefore, any time between the thirtieth and thirty-eighth hour of incubation would have sufficed for accurate results in this experiment.

The technic employed in this work was suggested by Professor Clark and modified to suit the experiment. It is as follows:

The egg is taken from the incubator and placed in a warm-box. The shell is sterilized at the point to be opened by wiping with a small cloth saturated with alcohol. As soon as the alcohol has evaporated, a small hole is scraped through the shell with the point of a scalpel using care not to puncture the shell membrane.

If a drop of warm water or Locke's solution is dropped upon the opening at this time, it will help to loosen the membrane from the shell, and will also prevent to some extent, long cracks and the breaking off of large pieces of shell. The shell is removed over an area about one centimeter or less in diameter and the shell debris washed off with warm sterile Locke's solution. The shell membrane is carefully stripped back with the forceps and the blastoderm exposed and flooded with Locke's solution. If the embryo is eccentrically placed, it may be necessary to rotate the egg slightly or remove more of the shell. The heart, if not visible, may be brought into view by pulling on the covering membrane with the forceps and turning the embryo slightly to one side.

The next procedure is cutting a small opening through the vitelline membrane and lateral plate and, catching the heart with the forceps, pulling it well out from the embryo and severing its connections, both anteriorly and posteriorly, with the scalpel or scissors. For this work I have used knives made from dissecting needles or by grinding down old scalpels, but I have found the small iridectomy scissors much easier to handle and more reliable. After removing the heart, the embryo is again covered with the sterile Locke's solution warmed to about 100 degrees Fahrenheit, the hole in the shell covered with a piece of isinglass, and sealed with a mixture of wax and resin (Rabaud). The egg is now returned to the incubator and allowed to develop further.

The operation is carried out in the warm-box at incubator temperature. If moderate aseptic precautions are observed there is little danger of infecting the embryo. The work is done under the binocular microscope, and is not difficult if the instruments are sharp, but if they are dull, especially the forceps, it is next to impossible to secure accurate results.

On several occasions, at stages of from sixteen to twenty-four hours incubation, I injured the embryo by burning with the cautery or by cutting sufficiently to prevent its further development. The extra-embryonic area, however, continued to grow in a manner similar to that in the experiments in which the

heart itself was removed, and the changes in its blood-vessels were similar.

The chicks were incubated varying lengths of time from twenty-four hours up to and including ten days after the operation. I was unable to keep any of the chicks alive longer than nine days, that is, about eight days after the operation. The mortality for chicks up to the end of the ninth day of incubation was about fifty per cent. After the egg has been incubated as long as desired, it is again opened and the vessels injected with India ink. The specimen is fixed in Bouin's solution, which may be applied to the chick while on the egg, or, preferably, after the embryo has been removed and flattened out on a slide. The specimens are stained faintly and cleared in oil of wintergreen (Spalteholz method), or, if desired, mounted permanently in damar. After being studied, the embryo has, in some cases, been embedded and sectioned.

Before injecting, a careful search is always made for remnants of the heart or any pulsating blisters, such as I will describe a little further on in this paper.

IV. DESCRIPTION OF THE EXTRA-EMBRYONIC VASCULAR SYSTEM OF EMBRYOS STUDIED

The appearance of the embryo at the stage of operation is shown fairly well in figure 1, which represents an embryo slightly beyond the operative stage At this period the blood cells have not acquired their hemoglobin and no vessels are visible under the binocular. The embryo appears to be floating free within the clear area pellucida, and the area opaca is apparent only as a wide milky-white band around the area pellucida. The blood vessels in the embryo proper of chicks of this age have been studied by Miss Sabin ('17) through injectiods of India ink into the aorta and larger vessels, and her results have already been referred to.

As previously mentioned, and as shown in figure 1, the vessels of the extra-embryonic region consist of a dense capillary net extending from the embryo to the sinus terminalis at the time the circulation begins. Anteriorly, the sinus terminalis breaks

Fig. 1[1] Camera lucida drawing of an injected, normal chick of 35 hours incubation, 16 somites, cleared in oil of wintergreen, viewed from above. The left half of the area is shown. Enl. $12\frac{1}{2}\times$.

[1] The chick shown in figure 1 was incubated 35 hours, but in development has reached the stage usually seen in chicks of about 38 hours incubation. It is, therefore, slightly older than most of the chicks at the time of operation. The ink was injected into the sinus terminalis and forced through the capillary net, or, as I have termed it in the text, the venous plexus of capillaries anterior to the embryo, to the rudimentary vitelline veins. Ink was also introduced into the heart. The heart pumped the ink back and forth into the sinus and vitelline veins for several minutes, but soon particles of the ink began to shoot through the arches into the right dorsal aorta. This was followed quickly by a rapidly growing stream of ink which moved forward as propelled by the pulsating heart and spread out into the capillary net at the termination of the dorsal aorta several

up into a plexus of venous capillaries through which, at a later stage, the blood from the sinus is returned to the heart. This venous plexus gradually becomes denser and evolves into the primitive right and left anterior vitelline veins as the heart is approached. Near the posterior end of the embryo, each aorta breaks up into capillaries, which are continued outward onto the extra-embryonic area by a dense capillary meshwork, in which there is as yet, no vessel marked out, as the omphalo-mesenteric artery—which develops here soon after circulation commences. There are the well-known 'clear' areas, without blood capillaries, surrounding the head region, others on either side of the body of the embryo between the anterior vitelline veins and the plexus in the omphalo-mesenteric region, in which, however, a single very narrow capillary is present in the specimen drawn, while there is a large oval non-vascular region including and surrounding the caudal undifferentiated end of the embryo. The capillaries bordering the clear areas next the body of the embryo are especially narrow, while near the border vein, particularly in the posterior portion of the area, they are very wide. This condition represents that immediately before the effects of circulation begin to be exerted, for, as stated in the note, circulation had already started on the other side, and a suggestion of the omphalo-mesenteric arteries could be made out there.

In normal development, soon after circulation commences, the omphalo-mesenteric arteries differentiate out of the capillary plexus, the anterior vitelline veins grow together anterior to the embryo, forming a single large vein, and, somewhat later, the posterior and omphalo-mesenteric veins develop. The fate of the border vein in normal embryos will be referred to later. These changes have been fully described by Thoma ('93) and Popoff ('94).

Let us now turn to the fate of the vessels deprived of the action upon them of the factors concerned with the circulation.

minutes before granules of ink began to appear in the left aorta. In a number of embryos studied, the circulation was invariably established upon the right side first. It will be noted that the aortae pass immediately into the capillary plexus in the posterior portion of the embryo, and that no large branches have as yet been formed out upon the yolk-sac.

In all of the chicks operated upon, with the exception of the ones that failed to survive the operation, the vessels of the extra-embryonic region were filled with red blood cells, which made them clearly discernible before the injection. These vessels, during the first two or three days after operation, were injected without especial difficulty, the injection mass spreading out uniformly from the point of injection. In later stages, however, increasing difficulty was encountered, the ink often going but a short distance. This difficulty proved to be due to the irregular narrowing and retraction of the vessels which occurs in later stages.

That the area vasculosa continues to grow and expand after the operation is certain, and the results of a number of measurements upon operated chicks are reported in tabulated form below as compared with normals at the time of operation and later.

SPECIMEN	LENGTH	WIDTH	REMARKS
	mm.	*mm.*	
Area vasculosa, 33 hours	20	18	Normal chick
Area vasculosa, 106 hours	38	29	Operated chick
Area vasculosa, 105 hours	70	60	Normal chick
Area vasculosa, $8\frac{1}{2}$ days	47	40	Operated chick
Area vasculosa, $8\frac{1}{2}$ days	130	70	Normal chick
Embryo, $4\frac{1}{2}$ days	6		Operated chick
Embryo, $4\frac{1}{2}$ days	7		Operated chick
Embryo, $4\frac{1}{2}$ days	13		Normal chick

It is apparent from this table that while there is a surprising amount of peripheral extension of the area vasculosa in the operated chicks, still the growth is less than half as rapid as in the normal chicks. The embryos showed a similar difference. A number of operated chicks measured approximately 6.5 mm. in length as compared with a normal embryo of the same age, which was 13 mm. in length.

The area vasculosa of the operated chicks did not always expand uniformly in each direction, as in the normal, but often grew out at certain points, usually posteriorly from the embryo, in advance of other portions. Figure 2 is a diagram of one of

these specimens. The effect of this irregular growth of the area is apparently shown in the shape of the capillary plexus—thus, in figure 13 the peripheral capillaries, formed by the breaking up of the border vein, have grown out so rapidly that the inner capillaries have assumed a radial appearance.

The general picture presented is shown in figure 3. The area vasculosa appears, at first glance, to form a homogeneous network of intercommunicating capillaries, extending from the embryo proper to the border vein, which forms a wide rim around the outside—(somewhat wider than shown in the illustration). Examined more closely, it is noted that, anterior to the embryo

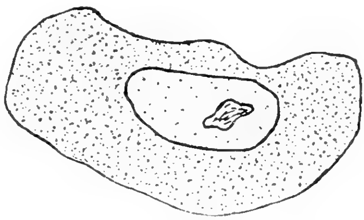


Fig. 2 A diagrammatic sketch (actual size) of the blastoderm of an operated chick of 73 hours incubation, showing the distorted shape of the area vasculosa.

there is a depression in the border vein, behind which a vessel, wider than most of the capillaries, extends toward the embryo—the anterior vitelline vein. It is also to be noted that, in the region surrounding the embryo, in the area pellucida, the spaces between the vessels are larger than in the area opaca. One looks in vain for any vessel which might be interpreted as omphalomesenteric artery, or posterior vitelline vein. To the left of the embryo there are two isolated blisters, and several blind ending vessels. Fuller reference to this will be made later.

Fig. 3 Camera lucida drawing of area vasculosa of an operated chick of 60 hours incubation (30 hours after operation). The capillaries in the area pellucida have begun to break up. *H*, a hole torn in the blastoderm when mounting, *X*, at this point the ink was forced out of the tiny vessels by too much pressure, and settling in the tissues, so obscured the capillaries that they could not be distinguished. *Ven. Vit. Ant.*, Anterior vitelline vein.

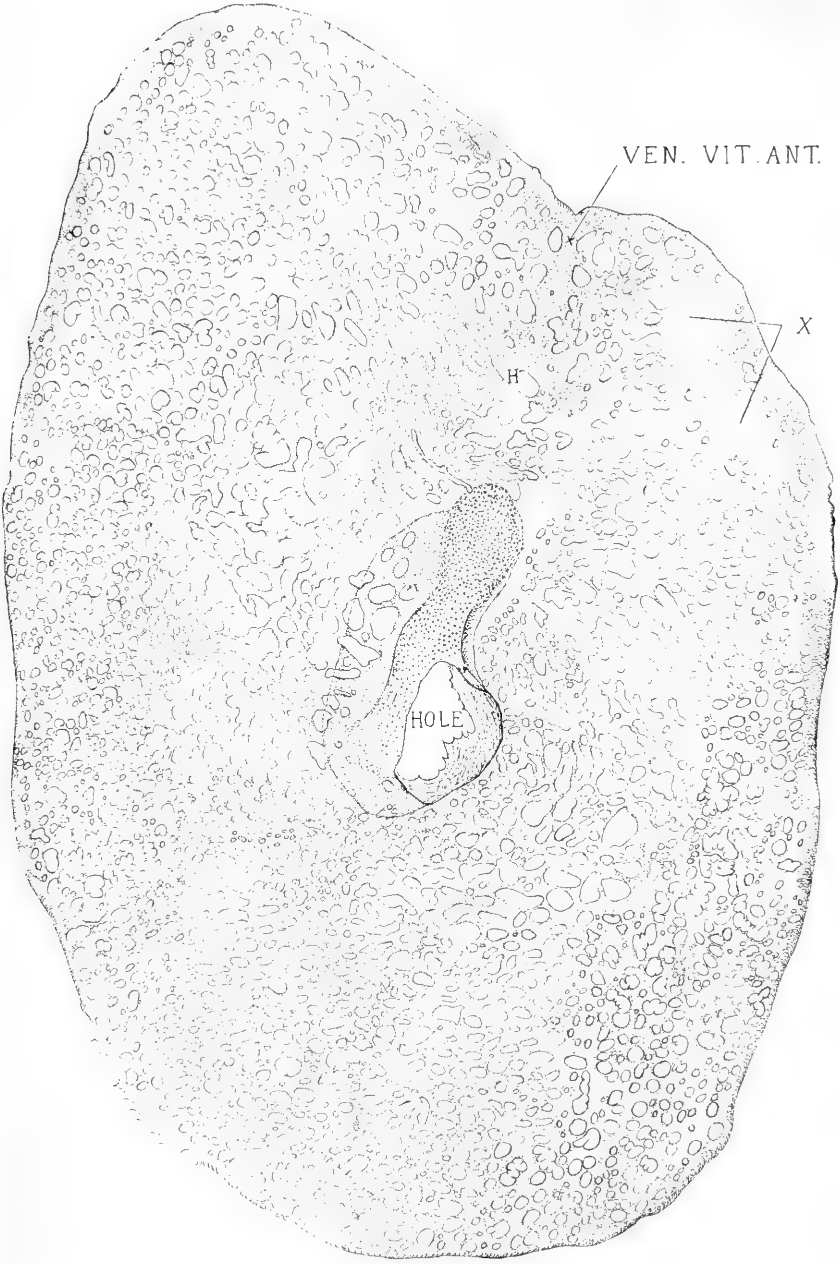

VEN. VIT. ANT.
X
H
HOLE

Figure 4 is a drawing of an unoperated chick which had been incubated 48 hours and had developed abnormally. The embryo appears as an oval membrane with two little splotches of protoplasm underneath it. There was no circulation, and a fairly uniform capillary plexus was present throughout the area, extending under the oval membrane. Only at one place—anterior to the membrane—is there any sign of a vessel larger than a capillary, which is very probably the rudimentary heart and anterior vitelline veins. There is no trace of any vessel which could be interpreted as omphalo-mesenteric artery. It will be noted that, as in figure 3, the continuity of the plexus has begun to be lost in the area to the left of—and posterior to—the embryonic rudiment.

In all the operated chicks which were studied, by injection and by combined injection and staining, there was never found any definite vessel which could be interpreted as omphalo-mesenteric artery or vein, or posterior vitelline vein. There were, however, certain characteristic changes, which were repeated in all specimens, some of which resembled the changes in normal chicks, and which will be taken up separately.

The anterior vitelline veins

As shown in figure 1, there is a space between the right and left anterior vitelline venous plexus at the time circulation commences. In normal chicks, as Popoff has shown, this space is encroached upon by the two plexus, until it is obliterated, the two veins fusing in the mid-line, forming a single vein, which, for a time, returns most of the blood of the extra-embryonic area. It was most interesting to find that in the absence of a heart-beat, the vessels in the proamnionic region continue to develop in an almost normal manner for a time. During the second day after the removal of the heart (third day of incubation) the right and left vitelline veins fuse across the mid-line anterior to the embryo, and a single vessel, which corresponds to the left anterior vitelline vein of the normal chick, is formed (figs. 5, 6 and 7, cf. also fig. 3). During the fourth day, however, this anterior vessel begins to break up into capillaries and soon disappears. This is the only vessel larger than a capillary that is

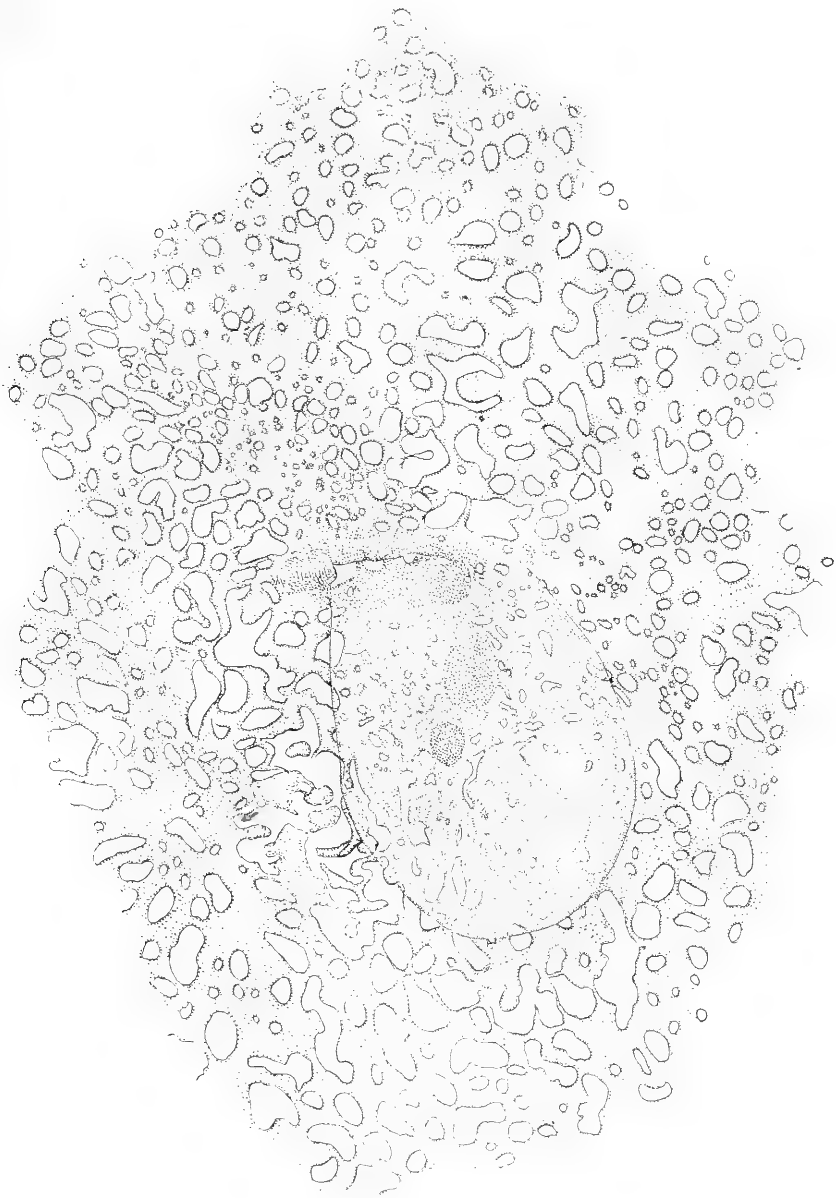
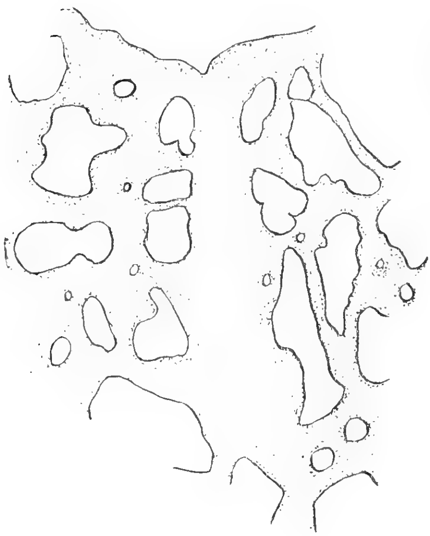


Fig. 4 Unoperated embryo of 48 hours incubation which developed without a heart. The sinus terminalis was present but is not shown in the drawing. Enl. 35 ×.

formed in the extra-embryonic region after the time when the circulation should begin, and with its disappearance and the breaking-up of the sinus terminalis into capillaries, which will be described in detail below, the condition becomes uniform throughout the extra-embryonic region.



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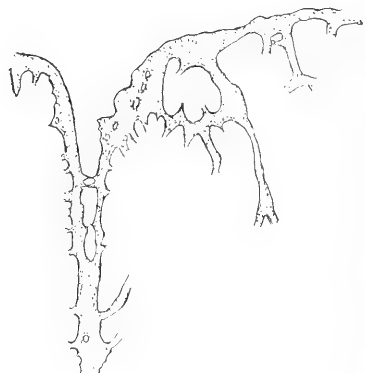

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Fig. 5 A camera lucida drawing of the anterior vitelline vein in an operated chick of 60 hours incubation (30 hours after operation).

Fig. 6 Camera lucida drawing of the anterior vitelline vein in an operated chick of 63 hours incubation (30 hours after operation).

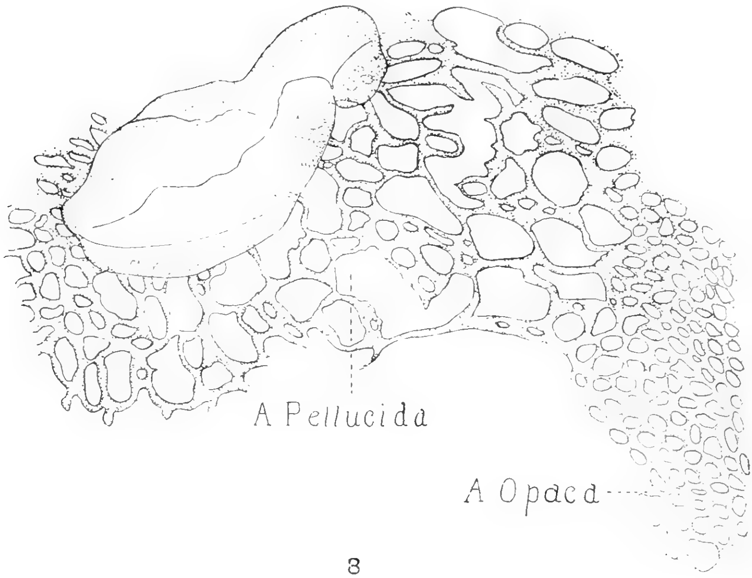
Fig. 7 A camera lucida drawing of the anterior vitelline vein in an operated chick of 80 hours incubation (48 hours after operation). The vessel has grown much narrower and is beginning to break up into capillaries nearer the embryo.

The vessels of the area opaca and the area pellucida

Soon after the operation a difference in the arrangement of the capillaries of the area pellucida and of the area opaca begins to appear, which becomes quite marked about the sixtieth hour of incubation (twenty-seven hours after the operation). In the former the spaces between the vessels are seen to be growing larger and the capillaries less numerous than in the latter (fig. 8). A trace of this is noticeable in figures 3 and 4. In the older specimens this condition is more pronounced. Figure 9 shows an embryo of four and one-half days in which the difference in the appearance of the capillaries is becoming decidedly marked and the two areas are separated by a rather definite border around the periphery of the area pellucida. In still older embryos the capillaries within the area pellucida become smaller in diameter and more scattered, and in embryos of seven or eight days (fig. 10) are broken up and appear as little streaks and puddles of blood, which are, in reality, isolated endothelial lined vesicles and spaces. A somewhat similar, though less intense, change occurs in the area opaca. Here the process is slower, and consists chiefly of the narrowing of many of the capillaries, some of which become solid and separate in the middle. Figure 11 shows a small portion of the area opaca in an eight day chick (seven days after operation), in which there are a great number of solid cords, some of which have broken in twain and begun to retract. The beginning of the process of narrowing and retraction of the capillaries in the area opaca is apparent by the fifth or sixth day, and is well demonstrated by the increasing difficulty of injection, which has been mentioned above.

The sinus terminalis

The sinus terminalis, or vena terminalis, as it is termed by Popoff, will now be considered. This relatively large embryonic vessel is present before the heart is formed (Lillie, '08, pp. 87–88). Popoff has described it as beginning to 'degenerate' (break-up) in chicks of forty somites, which would be somewhere around the eighty-fifth or nintieth hour of incubation, the breaking-up



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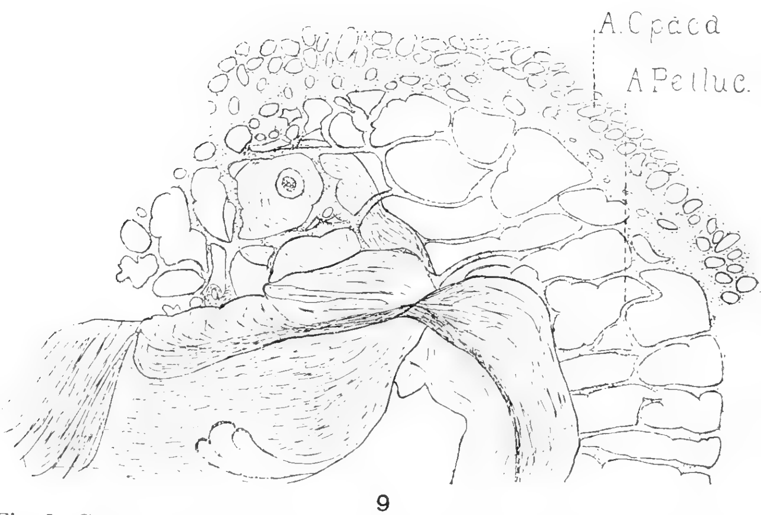

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Fig. 8 Camera lucida drawing of an operated chick of 90 hours incubation (57 hours after operation).

Fig. 9 A free-hand drawing of a chick of $4\frac{1}{2}$ days incubation in which the breaking-up of the capillaries in the area pellucida is quite pronounced. Operated at 33rd hour of incubation.

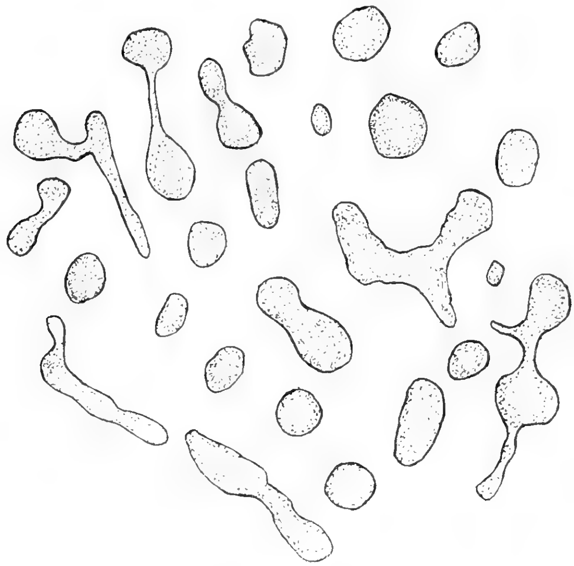


Fig. 10 A camera lucida drawing of a section of the area pellucida of a chick of 8 days incubation (7 days after operation) which shows small lakelets of blood formed by the breaking-up and retraction of the capillaries. Enl. 24 ×.

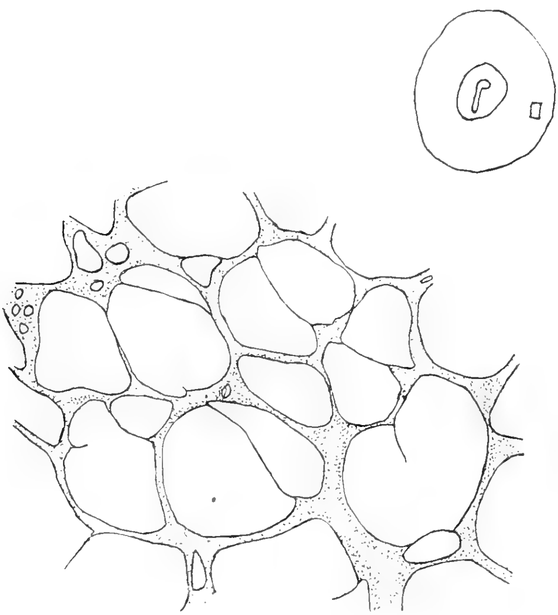


Fig. 11 Camera lucida drawing of capillary net in area opaca during eighth day of incubation. Operated chick. Enl. 52 ×.

continuing progressively until the sinus is resolved into capillaries by about the tenth day of incubation. I tested this out on a few normal chicks and found that the process begins about the time mentioned, but that it is often complete by the sixth day of incubation. I was much surprised to find that the sinus terminalis in the operated chicks followed this general rule and began to break up into capillaries at about the same time as the normal. Figure 12 shows the sinus at forty-four hours, before breaking-up has started. Figure 13 is from a chick of eighty

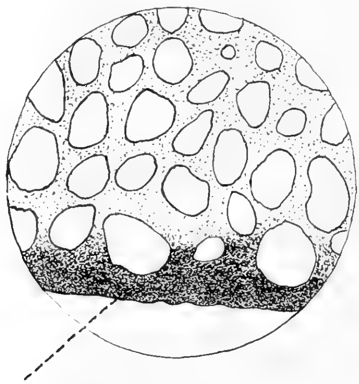


Fig. 12 A section of the sinus terminalis of a chick of 44 hours incubation in which the heart was destroyed by burning with the cautery in the region of the heart rudiment. Operated at twentieth hour of incubation.

hours incubation, and figure 15 is from a section of a chick one hundred and five hours old where the vessel has been replaced by a richly growing capillary plexus, which, judging by the peripheral sprouting, is highly vegetative. This was a somewhat extreme case and in the same embryo portions of the sinus had not yet begun to break up, although it had extended some distance from the embryo after the operation. Figure 16 is a camera lucida drawing of the sinus region of a normal chick of the same age. This drawing more nearly represents the conditions found in both normal and operated chicks of this age.

In operated chicks of seven or eight days of age the sinus terminalis had invariably broken up into capillaries, although the

process was often not complete. Figure 14 is a camera lucida drawing of a section of the sinus terminalis as it appeared in a chick during the ninth day of incubation. The vessel had disappeared over half of its length, and, on the side drawn, appeared so thin as to resemble a wide band of endothelium, sending out sprouts from its outer margin and gradually shading off into normal capillaries towards the inside.

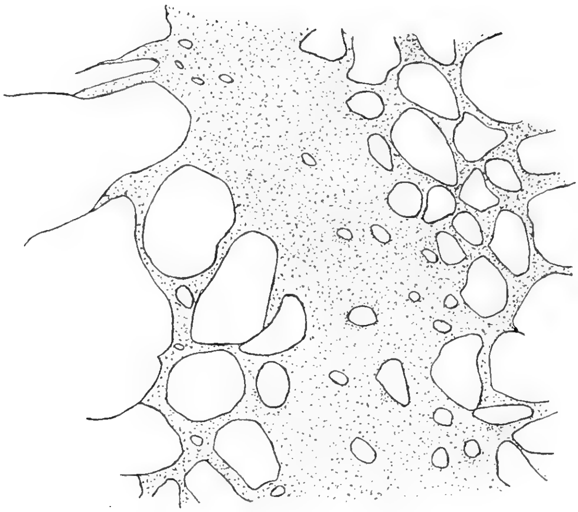
A condition somewhat similar to the ones described above was found in one of the eggs that I had been incubating as a normal. In this egg, (incubated six days) for some reason, the embryo had not developed normally and appeared as a small shapeless mass of protoplasm lying in the center of the area pellucida. The area vasculosa, however, was covered with a rich plexus of capillaries which were clearly alive and growing. There was no sign of a border vein around this plexus, and the capillaries were sending out numerous sprouts around their outer margin.

Fig. 13 A camera lucida drawing of a section of the area opaca in the region of the sinus terminalis which has broken up into capillaries. At this point, the capillaries have apparently grown. Operated chick of 3 days' incubation.

Fig. 14 Camera lucida drawing of the sinus terminalis in a chick during the ninth day of incubation (8 days after operation). Enl. 47 ×.

Fig. 15 Camera lucida drawing of a section of the outer border of the area opaca where the capillaries are highly vegetative and are sending out numerous sprouts. Operated chick of 105 hours incubation. Enl. 47 ×.

Fig. 16 Camera lucida drawing of the outer edge of the area opaca of a normal chick in which the sinus terminalis has broken up into capillaries. Drawn for comparison with figure 11. Incubated 107 hours. Enl. 44 ×.



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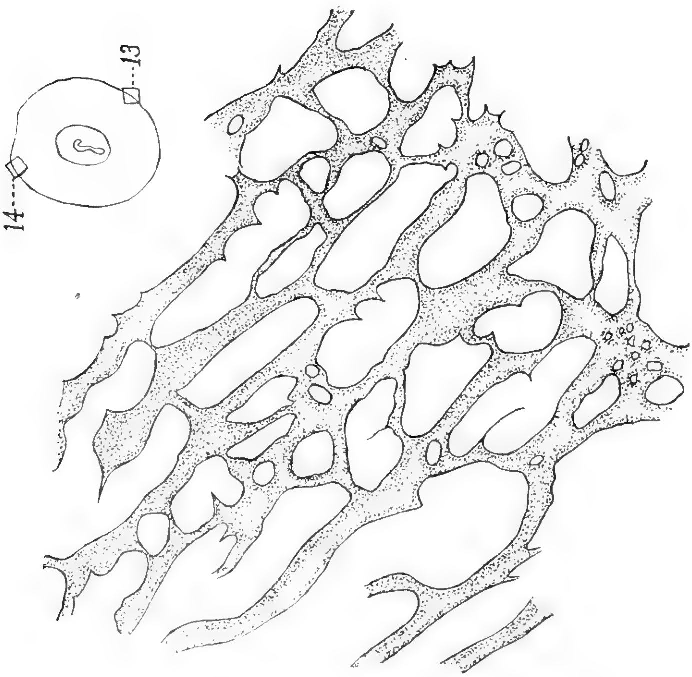

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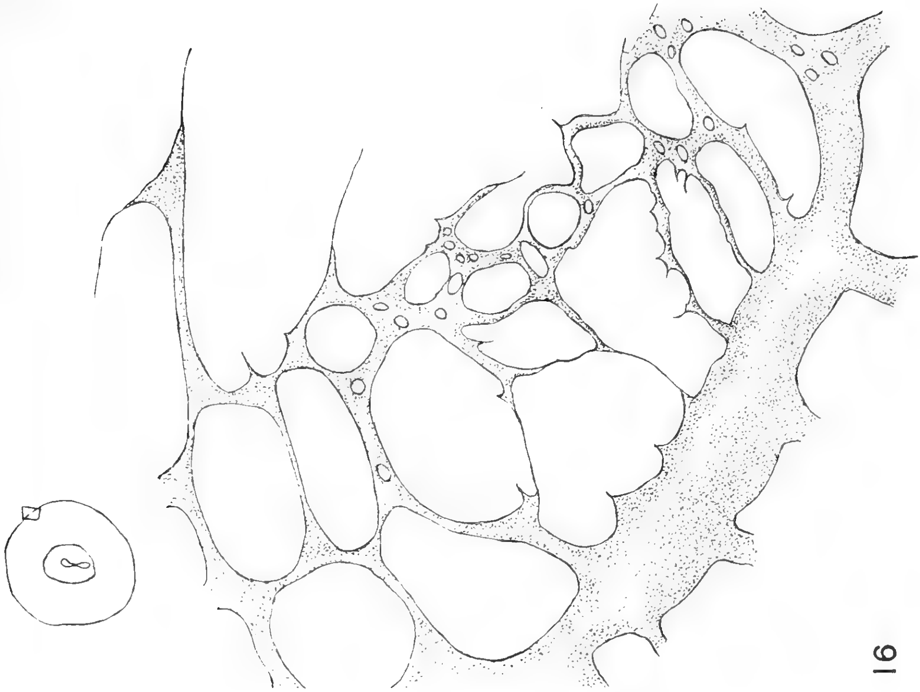

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V. A SHORT DESCRIPTION OF THE EMBRYOS

Although this problem deals only with the development of the vascular system, I will take the liberty to include a brief description of the embryo. Many of the embryos developed very abnormally, and in some it was impossible to distinguish the anterior from the posterior ends by the shape of the embryo alone, but in chicks where the heart was removed with a minimum of injury to the remainder of the chick, the embryo continued to develop for a time in an almost normal manner. The chick turned upon its left side, the amnion behaved as usual, the eyes were formed, and the wing buds appeared. The most abnormal feature was its failure to grow large. In no case did the embryo exceed 7 mm. in length after the removal of the heart. In a number wherein the heart was injured but not totally destroyed, especially in the operations with the electric needle, the size of the embryo was in proportion to the impairment of the circulation.

An examination of the embryos without hearts, in serial sections, showed very little that could be interpreted as normal. The neural tube and notochord were present, and the general outer contour had a normal appearance, but the embryo contained huge spaces beneath and to the side of the neural tube and notochord and connecting freely with the celomic cavity at many points. These relatively huge spaces are so packed with blood cells that it was often necessary to use the high power in order to tell the wall of the cavity from its contents. Mitoses were numerous both within the substance of the embryo and among the cells inside the open spaces. That several of these spaces were the remains of blood vessels was indicated, but they were very abnormal.

VI. MENTION OF RESULTS OBTAINED IN A NUMBER OF OPERATIONS WHICH WERE ONLY PARTIALLY SUCCESSFUL

The results obtained in a number of operations that were only partially successful are worth mentioning. In an effort to prevent the formation of the heart, I tried cutting through the lateral plates and dissecting around the neural tube of a number of

chicks that were opened before the heart had formed. This operation was often successful, while at other times the heart would later be found developing out in the area pellucida, separate and apart from the embryo or connected with it by a few capillaries. Figure 17 is a case of this kind. The small vesicle shown was pulsating at a normal rate when the egg was opened. By its dilatation blood was sucked in and with its contraction the blood was discharged again into the capillaries, the blood

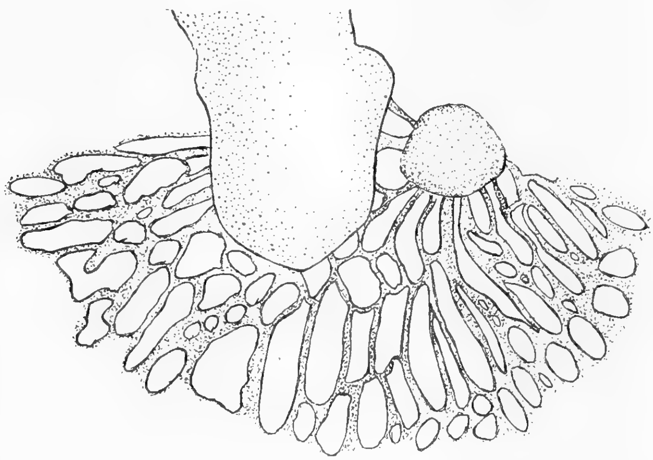

Fig. 17 A free-hand drawing of a chick in which the heart had not been destroyed, but developed out in the area pellucida. The appearance of the capillaries through which the feeble circulation flowed is illustrated. Incubated 3 days.

cells moving back and forth over about the same distance each time. That this process had some effect upon the formation of the capillaries directly connected with them is apparent from their radial arrangement as compared with adjoining capillaries to which the circulation did not extend.

After a similar operation, I found a pulsating vesicle in the area opaca very close to the sinus terminalis although not connected with the border vein. This vesicle was connected with a relatively huge blood vessel which extended parallel to the sinus terminalis for a short distance and then broke up into radial capillaries through which ink injected into the large vessel

passed quickly to the embryo. That this large blood vessel was formed from the capillary net by the functional activity of the pulsating blister can not be doubted. On another occasion I burned away all of an embryo anterior to the first somite. On opening the egg again I found one of these small pulsating vesicles which had developed from a remnant of the heart or the sinus venosus and was pumping away after the manner described for figure 17. I could mention a number of these freaks, but it would have no bearing upon the problem.

VII. DISCUSSION

It appears, at first, that a number of conflicting factors have been introduced into this experiment. Chief of which are the following:

1. The formation of the anterior vitelline vein after the time when the circulation should begin.
2. The failure of the large omphalo-mesenteric arteries and veins and the posterior vitelline vein to appear.
3. The peculiar behavior of the sinus terminalis.
4. The progressive peripheral growth and spread of the capillary net, in combinaton with the central process of retrogression.

That these factors were not due to accident, but to the enactment of certain simple laws of development that have been long recognized by certain leading scientists can readily be explained.

Roux has divided the formation of the vascular system into three stages, which are as follows:

1. A stage of primary differentiation in which the formation of the vessels is governed entirely by certain hereditary principles due to inherited characteristics.
2. A transitional stage between the first and third wherein the hereditary formation is gradually supplanted by a process of functional adaptation.
3. A stage in which the further formation of all vessels is due entirely to the mechanical forces acting through the circulation.

The early formation of blood vessels which takes place before the circulation begins is clearly not governed by the mechanical forces acting through the circulation, and their formation can be

accounted for only as a response to some hereditary principle which as yet is not understood and which most scientists of the present day are unwilling to believe exists. The early onset of the circulation has been a factor which has obscured the cause of the further development of the blood-vessels, and although it was known that there was some formation of blood vessels such as the heart and aorta before the establishment of a circulation, it has only been since the publication of Miss Sabin's work on the subject, that the extent of this early formation of blood-vessels has been appreciated.

It is now clear that by the time the circulation begins, the embryo has a primitive but complete system of blood vessels. With the advent of the circulation, which occurs about the thirty-eighth hour of incubation, we have the beginning of the second stage. In this stage the primary differentiation continues, but is gradually replaced by the functional or mechanical factors due to the heart-beat. In the normal chick, it is impossible to determine just how much of the further formation is due to the heart-beat, but in the operated chicks, the mechanical and functional factors are eliminated and the further development is due entirely to the continuance of the primary differentiation and laying down of blood vessels independent of mechanical influences. That this continues after the time when the circulation should begin is proved by the formation of the large anterior vitelline vein by the fusion of the two, right and left, vitelline veins. It is clear, therefore, that with the failure of the circulation to start at the usual time, the further development of the vascular system is not inhibited, but proceeds for a time in an almost normal manner, that is, it goes a little further and this relatively important embryonic vessel is laid down.

The failure of the omphalo-mesenteric arteries to develop proves that the formation of these vessels is due to mechanical forces acting through the heart-beat, and in the absence of these mechanical forces, due to the circulation, they are not evolved from the capillary net. In view of the fact that I have never at any time observed the slightest indication of the formation of these vessels in my operated chicks, I am at a loss to understand

the formation of the large vessels described by Patterson as forming in the region where these vessels are usually formed.

The peculiar cycle of development and retrogression through which the sinus terminalis passes would indicate a further example of this stage of primary differentiation persisting after the time when it is usually thought to have been entirely eliminated. It would also be interesting to know precisely how much further development takes place within the embryo in the absence of a circulation, but this presents a problem within itself, and cannot be taken up in this paper. Roux has stated that this early stage of primary development persists for a considerable length of time, perhaps up until adult life in the human. He thinks the closure of the ductus botalli which occurs at birth or a little later belongs to this stage.

That the mechanical forces very early assert their superiority in the extra-embryonic region is apparent, for with the breaking-up of the anterior vitelline vein and the sinus terminalis into capillaries the vessels of this region are reduced to an indifferent capillary plexus. This capillary plexus continues to grow at a gradually decreasing rate for a number of days after the time when it should become functional. With the failure of the circulation to start, we note, about the beginning of the third day of incubation, the regressive changes already mentioned as taking place in the area pellucida. This process is noted in all of the operated chicks, and also in the unoperated chicks that developed without circulation, and is plainly due to the processof retraction and degeneration which has been mentioned by Thoma.

In introducing his first histo-mechanical law, Thoma stated that the surface of a vessel wall ceases to grow when the blood-current acquires a definite rate. The vessel increases in size when this rate is exceeded, becomes smaller when the blood-stream is slowed, and disappears when it is finally arrested. The breaking-up of the capillaries in the area pellucida is clearly an application of the latter part of this hypothesis of Thoma's. In this instance, however, there is no circulation into which the contained blood can be pushed by the retracting capillaries, and as these small vessels are filled with blood cells, when they narrow

at certain points, break up, and retract, the blood cells are forced into little lakelets of blood surrounded by the endothelium of the retracted capillary which encloses the blood cells as a capsule.

This process continues progressively out over the area pellucida and area opaca, but does not advance so far as in the area pellucida. It manifests itself here by the narrowing of most of the vessels, and the solidification and retraction of some of them —processes which cause an increasing difficulty of injection. By this time, the sinus terminalis is also beginning to offer resistance to the injection mass. As this vessel is usually injected with ease in younger chicks, it is plain that it also under goes a process of narrowing which precedes its breaking-up into capillaries. The blood within these small vessels is contained under some pressure. This can be demonstrated by puncturing them with the injection needle which is usually sufficient to produce an extravasation of blood cells.

The general application of the histo-mechanical laws of Thoma to the factors observed in this experiment are too obvious to permit of much discussion. The failure of the omphalo-mesenteric arteries and vein to develop show that these vessels are entirely dependent upon the blood-stream for their development. Thoma observed this factor and studies the development of these vessels. He states that with the beginning of the circulation, a few channels in the capillary net are selected by the blood-stream in consequence of the general direction which is given to it by the position of the ends of the primitive aorta on the one side and of the venous ostia of the heart on the other. These channels contain the more rapidly flowing streams. They, therefore, dilate and become converted into arteries and veins. Thoma, did not, however, recognize the formation of any arteries or veins in the extra-embryonic region as due to other than mechanical forces.

The response of the capillaries to the feeble circulation maintained by the pulsating vesicles in the extra-embryonic region is another factor in support of his description of the formation of larger vessels from the plexus by a circulation.

Thoma states further that, after the beginning of the circulation, some channels which offer resistance to the flow of the blood,

and are thus very slowly traversed, atrophy, or disappear altogether. The progressive breaking-up of the capillaries which, in this experiment, is general throughout the area pellucida, and gradually extends to the area opaca, is clearly this same process on a much larger scale. The little lakelets of blood that are left being due to the inability of the vessels to entirely discharge their contents.

VIII. SUMMARY

In summarizing the chief factors that have appeared in this investigation, the following are the more important:

1. In chick embryos, in which the heart has been removed before the establishment of the circulation, the embryo and area vasculosa remains alive for seven or eight days after the operation. A limited amount of growth takes place in the embryo proper, while the area vasculosa spreads out over the yolk until it may reach a diameter of approximately 45 millimeters.

2. The development of the blood vessels in the area vasculosa is not entirely inhibited, but the process proceeds in a normal manner for a short period beyond the time at which the circulation usually commences. During this time there are formed in the extra-embryonic region vessels identical with the normal anterior vitelline veins, which fuse anterior to the embryo as in normal chicks, while the sinus terminalis passes through a cycle of development and regresson which markedly imitates the normal. On the other hand, other vessels which normally differentiate early, the omphalo-mesenteric arteries, as well as the omphalo-mesenteric veins, and the posteror vitelline vein, are not formed.

3. With the expansion of the vascular area, there is a continued formation of new capillaries; the property of sprout formation apparently being retained until death. After the third day, however, this is confined to the marginal portions of the area. Near the embryo, in the area pellucida, new formation has ceased at three days, and regressive changes commence; connecting capillaries are retracted, leaving isolated or nearly isolated endothelial blisters distended with fluid or blood cells. This regressive process advances gradually into vessels of the area opaca.

4. The general conclusion seems justified that, while certain large vessels such as the sinus terminalis and the anterior vitelline veins develop as a result of hereditary factors, and continue a normal development for a short time after the circulation starts, 'self-differentiation' of the vascular system is very limited, and the working out of most of the arteries and veins is dependent upon the mechanical factors concerned with the circulation of the blood.

I am indebted to Professor Eliot R. Clark for the assignment of this problem and valuable instructions during its investigation.

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VESTIGIAL GILL-FILAMENTS IN CHICK EMBRYOS WITH A NOTE ON SIMILAR STRUCTURES IN REPTILES

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THREE TEXT FIGURES AND FOUR PLATES

Since Rathke's discoveries in 1825, biologists have been interested in the branchial region of amniotes as supplying the most conspicuous example of the persistence of ancestral structures in the higher vertebrates. But no record has yet appeared, with the possible exception of Grosser's note on young human embryos, which reveals the presence of any structures on the gill arches of reptiles, birds or mammals, that can be interpreted as functional or rudimentary gill filaments. Rathke, indeed, described small, obliquely-placed plates (Blättchen) on the gill arches of mammalian embryos but in 1832 he stated that their irregular appearance made him very suspicious, and when he examined them more carefully, in a sheep embryo which had been in spirits some weeks, he became convinced that the leaflets were merely broken-down fragments of delicate branchial epithelium.

While studying the anatomy of the five-day chick and unaware of Rathke's earlier attempt to find gills in mammals, my attention was attracted to ectodermal proliferations, protruding from behind the hyoid arch, which seemed to be involved in the obliteration of the cervical sinus.[1] To Professor F. T. Lewis I am indebted for the suggestion that these projecting cell clusters might

[1] These were described briefly on page 18 of the following paper: "An anatomical study of the 13 mm. chick; a contribution to the comparative embryology of birds and mammals." (Manuscript deposited in Harvard College Library, June 1916.) See also Proc. Am. Assoc. Anat., Anat. Rec., vol. 10, 1916, p. 185; vol. 11, 1917, p. 329.

be brought into line with the gill filaments of amphibians and fishes. Subsequent study of older and younger chick embryos, together with the finding of similar structures in reptilian embryos, seems to warrant such a conclusion and the presentation of this material from a phylogenetic standpoint.

ORIGIN AND EARLY DIFFERENTIATION OF GILL-FILAMENTS IN THE CHICK

The history of these filaments, covering a period from the beginning of the fourth to the middle of the eighth day, occupies nearly one-fifth of the total period of incubation. Throughout this time the epithelium of the filaments themselves as well as the branchial epithelium which gives rise to them is characterized by the presence of what appear to be *degeneration vesicles.* These accompany, and thus may be said to register, an activity of the epithelium of which the filaments seem to be the fruition. They first appear in the entoderm, but later arise in the adjacent ectoderm, where they come to exist in greatest numbers. As early as the seventy-six hour stage (Harvard Embryological Collection, Series 2057, 6.8 mm., 76 hours; and Ser. 1953, 8.0 mm., 78 hours) the first of them may be seen in the posterior or posteromedial walls of the first four pharyngeal pouches at a time when the first three gill clefts have broken through and the fourth pouch touches the ectoderm (figs. 4 and 10). When complete, each vesicle is a clear spherical cyst, embedded in the epithelium, measuring some twelve to twenty microns in diameter, that is from two to three times the size of the erythroblasts in neighboring blood vessels. Each is surrounded by a wall of its own, comparable in thickness to a nuclear membrane, the whole enclosing pycnotic nuclei and cytoplasmic fragments in different stages of degeneration. Favorable sections, such as figure 25, indicate that these cysts result from the nearly simultaneous disintegration of adjacent epithelial cells. In places where two or more cysts have coalesced, the resulting structure often bears a superficial resemblance to capillaries containing corpuscles in the underlying mesenchyma, but the most careful search fails to reveal any direct relation between the two. Of interest, in con-

nection with the fact that the vesicles first appear in the entodermal walls of the gill clefts, is the observation, based on the work of Greil and others, that it is the entoderm of the pouches of frogs and toads, which initiates the process of gill formation. This it does by spreading out from the distal ends of the pharyngeal pouches on either side of a cleft until the ectoderm that covers the arches has become partly, and in some species completely, underlaid by a new layer of entoderm which has interpolated itself between the ectoderm covering the arches and the mesenchyma upon which the ectoderm formerly rested.

Following the first appearance of cysts in the chick, scattered vesicles may arise in the walls of all the pharyngeal pouches and in the ectoderm between the clefts on the outside. Toward the end of the fourth day they become most numerous in the ectoderm between the second and third clefts, where the degeneration is so rapid that pycnotic nuclei may appear anywhere in the epithelium without grouping themselves into vesicles and in sufficient numbers to give the epithelium a punctate appearance. Eventually most of them are crowded down into the underlying tissues. A second, less conspicuous concentration area occurs in the ectoderm between the third and fourth clefts. In frogs and toads it is the ectoderm of these two arches which evaginates to form the first external gills.

Before describing the further development of the epithelium, it is desirable to review the changes in the branchial arches as seen from the outside. Figure 4 represents these arches at the stage when the epithelial vesicles first appear. It will be noted that the hyoid arch is already larger than the others, that the third arch has become somewhat wedge-shaped and smaller, and that on account of this differentiation the branchial region as a whole has lost that regularity of arches and clefts so characteristic of early stages in all vertebrate embryos. In the next figure these differences are still further accentuated, the three-cornered hyoid arch dominating the whole region, while the posterior arches are depressed into a sinus below the general level of the neck and are correspondingly reduced. In certain fishes and amphibians it is the posterior margin of this second arch, now known as an

operculum or gill cover, which grows back over the sinus behind it, enclosing the third and fourth arches with their filaments in a branchial chamber. In the later stages of the chick this arch undertakes a similar backward extension, the significance of which was first appreciated by Rathke, the pioneer discoverer of gill clefts in amniote embryos.[2] In the wingless bird Apteryx (Parker), and in Struthio (Nassonow), the opercular fold to which this backward extension gives rise is said to be a very conspicuous object, much more so than in the other amniotes, although in all vertebrates, the hyoid is larger than any of the other arches.[3] Thus the persistence of this peculiar development of the hyoid arch through the whole vertebrate series is almost as striking as the persistence of the arches and diverticula themselves. In the light of this fact the presence of filamentous structures behind the operculum takes on added significance.

Returning to the discussion of figure 5, a careful examination of the third arch reveals a small hillock or mound just behind the point of the hyoid,—between the point and the third ectodermal groove. In fresh specimens observed under salt solution, this mound is distinctly whiter than the surrounding tissue and is the first external indication of the appearance of filaments. Serial sections of a slightly older stage than that referred to in

[2] "Am vierten und fünften Tage der Bebrütung kommen auf jeder Seite in der Substanz des Halses drei aufeinander folgende fast linsenformige Höhlen zum Vorschein, deren jede nach aussen und innen geöffnet ist. Die äussere Mündung der vordersten Höhle wird übrigens von einem Theile, der ähnlich dem Kiemendeckel der Fische ist, verdeckt." ('25)

In a much later publication ('61) the same author carries the analogy still farther. "Von dem zweiten Schlundbogen, in welchem sich ein Zungenbeinhorn ausbilden soll, wächst bald darauf, nachdem sich die vordeste Schlundspalte geschlossen hat, ein klappenartiger Fortsatz hervor, der die zweite Schlundspalte bedeckt und als eine Andeutung der *Membrana branchiostega* der Grätenfische betrachtet werden darf."

[3] Parker on Apteryx: "The backward extension of the hyoidean fold visible in the previous stages has increased so as to form a true operculum, which completely covers the third cleft, so that it is invisible in an external view. The fourth cleft . . . . lies immediately behind the operculum, and is very probably only exposed by the shrinking of the latter. . . . . The retention of so obviously amphibian a character . . . . appears to be a character of very considerable morphological interest."

plate 2 (see figs. 12 to 17 inclusive) show that the mound is an evagination of the thickened, vesiculated ectoderm covering the third arch, and contains a *mesodermal core*, thus almost reproducing the early formation of external gills in the amphibia. The lower end of it has already begun to develop filaments and later the upper end will give rise to tufts of cells (fig. 18). In the same embryo the ectoderm of the fourth arch forms an evagination, which however, is solid, and in this specimen much smaller, tending to fuse with that from the third arch (fig. 15). Owing to the rapidity with which the region behind the third arch is being flattened out, the evagination on the fourth arch, which has been observed in several embryos, has only a transitory existence of its own. As the operculum extends backward all of the third arch except the mound becomes covered over, while the mound itself gradually assumes the shape of a wedge, with filaments at its downward directed point, as indicated in figures 6 and 19. As the hyoid arch continues its backward growth during the fifth day it fuses with the third arch in such a way as to carry with it on its under surface the tufted epithelium at the lower end of the wedge, so that from now on, the filaments of this region of fusion will appear to come from the under surface of the operculum (figs. 19 and 23). By the beginning of the sixth day the whole edge on each side has become differentiated into a ridge coextensive with the lateral margins of each operculum (fig. 7). Serial sections (fig. 24) show that the individual filaments borne by the ridge are solid outgrowths of the epithelium, honeycombed with degeneration vesicles. With the appearance of this pair of ridges the first half of the life-history of the filaments may be said to have been completed.

While this differentiation of filaments has been going on at the margins of the hyoid arches, the ventro-medially directed portions of the two opercular processes have united to form a single band of tissue slightly overlapping the pectoral body-wall and extending across the ventral surface of the neck from side to side,—the homologue of the *membrana branchiostega* according to Rathke. From now on, the fused hyoid arches may therefore be referred to as a single structure, the *plica opercularis*, pos-

sessing two *lateral margins*, each fused to the side of the neck and bordered by a line of filaments, and a single *pectoral margin* whose free edge is directed posteriorly. A notch at its middle point (*incisura opercularis*) indicates the place where the two arches originally united, and a *tubercle* on each side of the notch still further accentuates the paired origin (fig. 1). In later stages the free, overhanging, pectoral margin becomes more and more encroached upon by the lateral margins, as its under surface progressively fuses with the surface of the neck, from the sides to the mid-line. As these lateral zones of fusion pass slowly inward they carry the lines of filaments with them so that these likewise come to lie successively nearer the mid-line. Meanwhile the pectoral wall below the opercular fold has developed a pair of surface markings of its own which begin to shift their position from the sides to the mid-line at the same time and rate as the lines of filaments and zones of fusion above. Eventually these markings come to form part of a median ridge extending from the region below the operculum to the umbilicus. In addition to their migration these pectoral markings or ridges exhibit a further, albeit superficial, resemblance to the filaments in that the cell-proliferations of which they are made often contain scattered degeneration vesicles and pycnotic nuclei, but to a much lesser extent than obtains in the branchial epithelium. Because the pectoral ridges and lines of filaments are thus found to possess these common features it becomes necessary to establish the identity or the disparity of the two sets of structures, the one on the neck and the other on the pectoral wall,—hence the following digression.

CHANGES IN THE PECTORAL WALL CORRELATED WITH THE LATER DEVELOPMENT OF FILAMENTS AND OPERCULUM

During the latter part of the sixth day and the beginning of the seventh, four different sets of structures make their appearance in the pectoral wall, all of which are represented in figure 1: a pair of pectoral grooves (*sulci pectorales*); 2) a pair of pectoral ridges (*cristae pectorales*); 3) a pair of mesothelial ridges (*cristae mesotheliales*) and 4) a median epitrichial ridge (*crista epitrichialis*).

The *pectoral grooves* first appear about the middle of the sixth day and at once delimit a roughly triangular area whose base coincides with the intersection of the neck and breast, and whose downward directed apex lies just above the umbilicus. At first the area enclosed by these grooves is transparent throughout, revealing the outlines of the heart beneath. But almost immediately the basal third becomes vascular and much thicker than

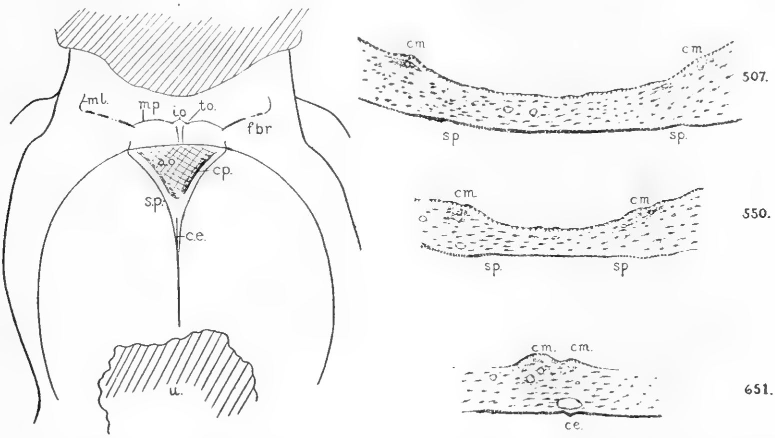


Fig. 1 Sketch of the markings on the pectoral wall of a seven-day chick (H. E. C., Ser. 2076; 17.3 mm.; 6 days, 7 hours) together with three transverse sections of the pectoral wall of the same embryo, × 42 diam. *507*, section at level of *c.p.*; *550*, section at level of *s.p.*; *651*, section at level of *c.e.*, *m.l.*, and *m.p.*, lateral and pectoral margins of opercular fold; *i.o.*, opercular notch; *t.o.*, opercular tubercle; *f.br.*, row of branchial filaments; *a.o.*, opaque area; *c.p.*, pectoral ridge; *s.p.*, pectoral groove; *c.e.*, epitrichial ridge; *c.m.*, mesothelial ridge; *u.* umbilicus. Compare with frontal section of pectoral wall in figure 20.

the apical portion which retains for some time its non-vascular and transparent character. In fresh specimens the upper portion exhibits a semi-opacity somewhat similar to that of ground glass. It is this part which at the beginning of the seventh day gives rise to a series of superficial evaginations which may appear anywhere in this area, but are chiefly ranged along the medial borders of the pectoral grooves. Ultimately those of a side become numerous enough to form a pair of *pectoral ridges* which in their later development, as previously noted, exhibit a super-

ficial resemblance to the filaments when seen in section. About the same time a pair of sub-surface lines may be seen through the translucent wall underlying each groove. A study of serial sections (H. E. C., Ser. 2076; 6 days, 7 hours; 17.3 mm.; fig. 1) proves them to be *mesothelial ridges* projecting into the pericardial cavity.

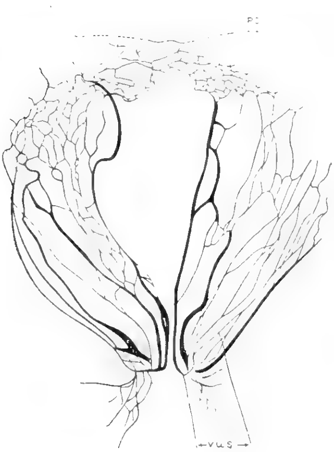
Hardly have these lateral grooves and ridges appeared than they begin to shift their position from the sides of the embryo to the mid-ventral line. This takes place in such a way that the legs of the triangle first approach each other in front of the umbilicus and thereafter successively forward of that point, thus resulting in an apparent ascent of the apex of the triangle. This progressive movement is recorded on the median line by an *eruption of epitrichial cells* which follows the retreating apex up the pectoral wall until it reaches the surface ridges described above. Thus a Y-shaped ridge is produced on the ventral surface of the embryo the upper arms of which, the two surface ridges, form a broad angle, and the lower arm of which, the epitrichial ridge, extends to the umbilicus. Micrometer measurements of selected embryos indicate the rate at which the two upper arms are coming together. In an embryo of five days, twenty hours (17.5 mm.) the distance between the upper ends of the ridges is 1.64 mm.; while in an older stage (6 days, 3 hours; 17.3 mm.) it has been reduced to 0.91 mm.; and in a still older embryo (6 days, 18 hours; 19.5 mm.) to 0.31 mm. The shifting of these superficial ridges also keeps pace with the shifting of the operculum and filaments to be described later, so that if the ridges were continued upward at any given stage they would strike the tufts of filaments above. In all cases, however, the two structures are separated by an appreciable area of the neck. Eventually the surface ridges become heaped up in the median line thus constituting, with the epitrichial proliferations, a continuous median ridge from the umbilicus to the neck. In its upper end the evidence of its paired origin is visible for some time and as a whole the ridge persists for a number of days even to the time when it becomes elevated upon the developing feather papillae (H. E. C., Ser. 1967; 11 days, 0 hours; 31.0 mm.). A similarly placed

median ridge has been observed in a human embryo of 45.0 mm. (H. E. C., Ser. 2079) and in a dog embryo of 14.0 mm. (H. E. C., Ser. 2052), but I have been unable to discover any clue to its origin in these animals. It is possible that in mammals, only the last stages of the process are visible.

An examination of the inner surface of the pectoral wall shows that an approximation of the two mesothelial ridges is also taking place (fig. 1). In sectioned embryos each ridge was found to contain one or two small veins although no blood vessels could be detected in the area between the two ridges in their lower extent. This suggested a study of injected embryos which has strikingly substantiated the shifting of tissues in the pectoral wall.

The displacement of veins in the pectoral wall. In a five-day chick that portion of the body wall which covers the heart is entirely free from blood-vessels. On the margins of this roughly triangular area lies a capillary network, continuous with that which fills the rest of the *membrana reuniens* (Rathke's term for the thin somatic wall which originally covers the abdominal and thoracic viscera). This appears to be growing into the wall over the heart much as capillary-nets elsewhere invade non-vascular regions. By the middle of the sixth day (injected embryo 5 days, 7 hours; 12.4 mm.) this network of the membrana reuniens has resolved itself into a series of radial veins converging upon the umbilicus from the myotomes. Those immediately adjacent to the non-vascular triangle (that is, under the pectoral grooves) converge upon the umbilical vein of their respective sides at the point where it enters the septum transversum, on either side of the apex of the heart. From now on, the non-vascular area over the heart will become more and more circumscribed, not by the ingrowth of new vessels (except in the uppermost part which is congruous with the opaque area previously described, where a capillary net grows down from the cervical region) but by a shifting of marginal veins already formed. These swing in on two pivotal points, the points referred to above, where the umbilical vein of each side enters the septum transversum. These radial venules are not straight lines but present a convex

surface to the non-vascular triangle, so that as they swing in they first meet in the median line under the point where the epitrichial ridge first appears, and thereafter progressively anterior to this point. In an embryo of 15.5 mm. (fig. 2) they are just reaching the mid-line for the first time. In an embryo of 18.5 mm. (injected specimen 6 days, 0 hours), they have moved



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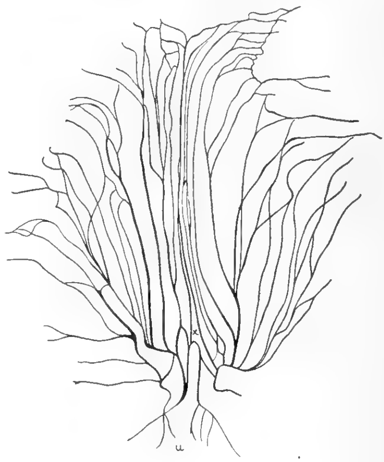

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Fig. 2 Blood vessels in the pectoral body-wall of a 15.5 mm. chick. Camera lucida drawing of injected embryo ji (6 days, 0 hours) × 15 diam. Note the non-vascular area in the center and the capillary plexus above it which has grown down from the cervical region. *p.o.*, opercular fold; *v.u.s.*, left umbilical vein.

Fig. 3 Blood vessels in the pectoral wall of a 20.6 mm. chick. Camera lucida drawing of injected embryo ka (7 days, 2 hours) × 15 diam. Note the disappearance of the non-vascular area and the direction of the blood vessels as compared with those in figure 2. *u.*, umbilicus; *x.*, median line, on either side of which are the right and left sets of marginal veins.

in upon the mid-ventral line as far up as the point where the epitrichial ridge meets the surface ridges, (the region of the truncus aorticus). Finally in an embryo of 20.6 mm. (fig. 3), the area over the heart is completely filled with parallel longitudinal veins extending from the neck to the umbilicus. Thus in forty-eight hours the original marginal veins of each side have described an arc of 45°.

As far as one can judge from the meagre description of the region in mammals, it seems probable that the pectoral wall is vascularized by a different method than that which obtains in the chick. Evan's figure of a fifteen millimeter pig embryo suggests that the pectoral wall is invaded by the same capillary net which grows down from the cervical region in the chick, but in this animal continues until it reaches the umbilicus. Kölliker's figure of a cow embryo of about the same stage suggests that this area is supplied by the capillary net which grows in from the marginal veins. Mall describes the condition in man as follows:

> I have in my collection a well-preserved human embryo (no. LXXVI) (22 days), in which the membrana reuniens is filled with a plexus of veins much like that in the cow's embryo . . . . The ventral wall of the heart near the liver contains no vessels, while the membrana reuniens covering the upper end of the heart is filled with a plexus of vessels which communicate with the capillaries of the mandibular arch.

The study of the blood vessels thus confirms the testimony of the superficial markings, that there is a considerable shifting of body-wall tissues from the sides of the embryo toward the mid-ventral line,—this in advance of the later invasion of pectoral muscles, nerves, dermal and skeletal parts which enter into the formation of the definitive pectoral wall. Whether there is anything of this preliminary movement in other amniotes is difficult to determine from the evidence at hand. But certainly in chicks of the sixth and seventh days it is possible to demonstrate a medial displacement of tributaries of the umbilical veins and a shifting of an internal and external set of ridges. Although I have been unable to find in the literature any record of such ridges or grooves as have just been described, there is some evidence that the older embryologists who discussed this region in birds and reptiles saw something of this early shifting of tissues. Thus Rathke, the first to maintain that the muscle and skeletal elements of the breast wall did not arise in situ, believed that these structures grew in from the sides of the embryo in such a way as to push ahead of them the thin somatic wall which originally covered the thoracic and abdominal viscera, eventually causing the membrana reuniens to disappear completely (allmälig ver-

schwinden). Remak, following Reichert, believed that the new elements did not displace the original wall but were merely incorporated into it as they grew in. ("Die sekundäre Bauchwand entsteht aus der Verschmelzung der unsprünglichen Bauchwand mit der hervorwachsenden Entwickelungsproducten der Urwirbel."). Although my own observations have resulted in the finding of new manifestations of a migration of tissues in the body-wall it is still very difficult to analyze the nature of the movements. It may be that at a certain stage in the development of the wall that part of the membrana reuniens which lies over the breast is being pushed in toward the median line by the faster growing lateral parts and reduced to a narrow zone,—the slack, so to speak being taken up by the increasing thickness of the membrana and by the protrusion of certain external and internal ridges. Or it may be that this early shifting of structures is more in the nature of a preliminary growth-wave which is passing from vascular to non-vascular territory.

The difficulty of ana'yzing these changes, however, does not derogate from the conclusions which we may now form concerning the relation of the pectoral ridges and the branchial filaments, —to ascertain which this study of the pectoral region was first undertaken. As has been stated, both sets of structures are evaginations of surface epithelium, both move in toward the median line at certain stages in their development and both exhibit some degree of epithelial degeneration. But beyond these superficial resemblances there is nothing to indicate the existence of any genetic relation between them. At no period in their development are they in continuity nor do they even resemble each other in histological appearance, unless it be at the end of the seventh day, when the filaments have been crowded in upon the tubercles just prior to their disappearance. The differences which they exhibit may be summarized in the following paragraph.

The epithelial evaginations which constitute the ridges develop from a broad zone of thickened ectoderm situated on either side of the pectoral wall. The margins of each zone grade imperceptibly into the adjacent ectoderm so that its limits are never

sharply defined. Apparently the limits vary considerably in different embryos. This variability may be said to characterize the whole development of the ridges,—a marked contrast to the regularity with which the filaments develop. As each zone shifts its position medially, scattered pycnotic nuclei appear between the epitrichial and basal layers of the epithelium and diffuse tufts of cells arise on its surface. The crests of these evaginations form a low ridge which becomes the more conspicuous the nearer the ridge approaches the mid-line. The filaments, on the other hand, grow out from a narrow strip of epithelium situated on the lateral margin of each operculum, a germinative zone which represents the fusion of the posterior wall of the hyoid arch with the ectoderm of the third and fourth gill arches. The filaments thus arise from a specific epithelium,—a portion of the branchial membrane, the whole of which is characterized at an early stage by the presence of degeneration vesicles. The filaments grow out of the region where the cysts occur in greatest numbers and are themselves honeycombed with vesicles. Furthermore, they do not differentiate into diffuse clusters of cells but into a row of more or less distinct evaginations. Again, the 'life cycle' of the filaments is staged from two to three days earlier than that of the ridges. The branchial evaginations first arise in situ and only later become involved in the movements of the ventral body wall, whereas the pectoral ridges are in process of migration when they first appear. Thus one is lead to consider whether the ridges are not more intimately connected with the movements of the body wall than are the filaments, if indeed they are not products of that movement. For as the ridges approximate each other they become heaped up in the midline to form a single structure which persists three days after the filaments have disappeared, whereas the latter never meet in the mid-line but maintain their identity as paired rows of individual filaments to the end. It may be that the pectoral ridges represent the survival of some similarly placed outgrowths in a lower vertebrate, but in no other animals, so far as I am aware, do any such structures exist. For the present, then, it seems more reasonable to define the ridges as local manifestations of migra-

tion in the pectoral wall. But whatever interpretation they may receive it is evident that they belong in a different category from the filaments.

LATER DEVELOPMENT OF FILAMENTS AND OPERCULUM

The description of the early development of these structures has been carried to the point where the opercula of the two sides have united to form a swollen band of tissue across the ventral surface of the neck (the plica opercularis) the posterior edge of which (the margo pectoralis) slightly overlaps the pectoral wall. On the lateral margin of the fold (the margo lateralis), a line of filaments has been formed, which is separated from the one on the other side by the whole width of the neck. This is the condition at the middle of the sixth day when the opercular fold has reached its maximum length of two and a half to three millimeters. From now on, the neck will increase in diameter as the fold undergoes reduction. This process consists in the fusion of the under surface of the margo pectoralis with the ventral surface of the neck, so that its form is changed from an overhanging fold of tissue to a mound, which in turn flattens out and eventually disappears. The striking feature of the whole process is that it proceeds from the sides to the midline, at the exact rate and at the same time that the pectoral ridges and marginal veins are moving across the face of the pectoral wall. The rate of fusion is easily gauged by measuring the decreasing distances between the medial ends of the two rows of filaments as they are borne along on the advancing wave. In all cases they move synchronously with the structures below. Thus in an embryo of 5 days and 20 hours (17.5 mm.) the unfused or overhanging portion of the plica, measured by the distance between the filaments of the two sides, is 1.45 mm. In the next seven hours it has been reduced in length to 0.55 mm. (embryo of 6 days, 3 hours; 17.3 mm.), and in the next fifteen hours to 0.23 mm. (embryo of 6 days, 18 hours; 19.5 mm.). The entire opercular fold including both overhanging and fused portions of the two sides measure as before some two and a half to three millimeters although the fused portion is in process of sinking into the neck and disap-

pearing. By this time the projected width of the neck at this level measures some three and a half millimeters. In round numbers, during the twenty-four hours following the maximum development of the operculum the unfused portion has been reduced by eighty per cent of its former length while the neck has added twenty per cent to its circumference. By the beginning of the eighth day the united opercula have become reduced to a pair of tubercles on either side of the mid-ventral line which are themselves on the point of being incorporated in the neck.

While the opercular fold has been undergoing a decline, the filame ts have reached their maximum development and have likewise entered a period of decline which is completed with the disappearance of the tubercles. In the beginning it was stated that the filaments were solid outgrowths of cel s arising chiefly from the ectoderm covering the third branchial arch; that these filaments first appeared at the lower part of the evagination of that arch; then peripheral to this point as the mound assumed a wedge-shape and the wedge became compressed into a filament-bearing ridge, about half a millimeter in length. Such is the condition at the middle of the sixth day, at a time when the plica opercularis is coextensive with the width of the neck, and when the pectoral grooves and other evidences of the median migration first appear in the wall below (fig. 7).

As the zone of fusion between the under side of the opercular fold and the neck moves inward from its original position at the junction of the lateral and pectoral margins of each side, the row of filaments is carried with it. Concurrently each row increases its length until it reaches a maximum extent of nearly a millimeter, and numbers some eight to a dozen separate filaments. These are often irregularly arranged and are sometimes grouped into two parallel rows. Starting with the medial end the small ones with which the line begins pass abruptly into large-size filaments which continue from a third to half way across the line. Lateral to this medial portion there are usually gaps in the line and the different members vary in height, tending however to become somewhat smaller as the lateral end is approached. At exactly what point new filaments are added to give the line

its maximum extent or how the movement of the line as a whole across the neck is accomplished, is difficult to determine. One is inclined to believe that the medial migration is an apparent rather than a real movement, brought about by the addition of new members to the medial end of the row, this end representing an advancing growth-zone superimposed upon the advancing zone of fusion between the operculum and the neck. In favor of this hypothesis is the fact that the medial half of the row exhibits the greatest solidarity, that the large medial filaments are the ones that are usually branched (fig. 20), and that the lateral members are the ones which drop out as the total length of the line diminishes. There is the other possibility, however, that the line as it stands is carried bodily inward, new filaments being added laterally (the order of formation in the earliest stages), or possibly interspersed among the old ones as the line is drawn out. Following the period of maximum development during the seventh day, the lateral members of the row gradually flatten out until, as the line approaches the center, only a few of the medial filaments on each side remain (fig. 9). By the beginning of the eighth day both the opercular tubercles and filaments have been absorbed into the neck.

During this shifting of the rows of filaments from the sides to the mid-line the under surface of the pectoral margin of the opercular fold between the right and left zones of fusion has given rise to a *new* line of filaments,—abortive structures which are so small that they cannot be made out with the naked eye. In fresh specimens, however, the margo pectoralis has that pearly lustre characteristic of the marginal filaments. These abortive filaments can just be made out with certainty in sections of seventh-day embryos (H. E. C., Ser. 1950; 6 days, 2 hours; 16.0 mm.) (H. E. C., Ser. 2075; 6 days, 1 hour; 17.0 mm.), where they appear as low sprouts of cells on the under surface of the opercular fold. The largest of these are to be found on the tubercles which lie on either side of the notch. As the marginal filaments move in, they push this secondary line ahead of them and at the end often form with these a confused tuft of cells just prior to the final disappearance of both filaments and operculum

In figure 9 the primary and secondary series have maintained their identity to the end. In reviewing the origin of both series it will be seen that the posterior wall of the hyoid arch is potentially a filament-bearing surface as well as the walls of the third and fourth branchial arches. This is in accord with what we know of conditions in gill-bearing vertebrates.

By way of a *summary* the history of these vestigial gill filaments in the chick may be divided into six stages: 1, the appearance of *degeneration vesicles* in the branchial epithelium; 2, the *concentration* of these in the ectoderm covering the third arch and, to a lesser extent that covering the fourth arch; 3, the thickening of the ectoderm of these two vesiculated areas into tufted epithelial mounds, and, in the case of the third, an apparent *evagination of the ectoderm* with a mesodermal core; 4, a gradual *differentiation* of these areas (now crowded into one and fused with the sides of the backward growing operculum) into a transverse line of filaments on each side of the neck; 5, a progressively medial *displacement* of this line, correlated with the medial migration of structures in the pectoral body-wall; 6, a rather rapid *reduction* of this line and the eventual suppression of both filaments and operculum.

GILL FILAMENTS IN REPTILES

Although the branchial region of reptilian embryos exhibits some measure of transition between the higher amniotes and the gill-bearing vertebrates it is much more nearly akin to that of birds than to that of any other group. Particularly is this true with regard to the development of the hyoid arch, where in turtles, lizards, and alligators (as in birds) the opercular processes of the two sides unite to form a conspicuous fold of tissue across the ventral surface of the neck which persists long after all trace of the other gill arches has disappeared. Similarly, vestiges of filamentous structures behind the hyoid arch are to be found in at least three of the main groups of reptiles, although in a more transitory and less conspicuous form than obtains in the chick. That they are not developed to a higher degree in the former may be explained by the fact that living reptiles are themselves a

modern and highly specialized group; and that the degree to which retrograding structures are developed does not necessarily correspond to the rank which the possessor of these structures holds in a graded phylogenetic series. The fact that these structures are present at all in reptilian embryos greatly increases the significance of the better developed filaments in the chick embryo.

In discussing the conditions in reptiles it should be borne in mind that considerably less material was available than in the study of the chicks where some seventy embryos between the sixth and ninth days of incubation were examined. Of the four reptile series in the Harvard Collection, which are sufficiently extensive to afford a fairly complete picture of the development of the branchial region, three of them, Lacerta, Eutaenia and Chrysemys, show epithelial outgrowths behind the hyoid arch which are identical with the filamentous structures found in the chick. The first of these, *Lacerta muralis*, presents a more primitive branchial system than is found in birds, five well-spaced ectodermal grooves being visible from the outside at an early period. Later in its development the operculum fuses with the region behind the fourth arch in such a way as to form a peribranchial chamber into which portions of the third and fourth arches with their respective aortic trunks freely protrude (H. E. C., Ser. 813; 6.4 mm.). Still later, when the branchial chamber has become obliterated, small epithelial proliferations appear from underneath the operculum in the region of its fusion with the posterior gill arches (H. E. C., Ser. 811 and 812; 7.4 mm.). Although they have but a transitory existence they occur at the same relative time and place as the filaments in the chick, with the difference that the filaments in the birds appear on the third and fourth arches prior to their fusion with the operculum as well as afterwards. In *Aristelliger praesignis* this is apparently reversed; the epithelium of the third arch is very much thickened just prior to fusion with the operculum, but thereafter no filaments are to be observed, as if a somewhat premature fusion, as compared with conditions in other embryos, had inhibited the epithelial proliferation which had already started (H. E. C., Ser.

1884; 4.9 mm.). The same is true of *Sphenodon punctatum* (H. E. C., Ser. 1491; 7.9 mm.).

In contrast with these lizards the snake *Eutaenia* presents a very interesting condition. So great is the lengthening process to which the body as a whole is subjected that the gill arches and clefts are obliterated by being drawn out instead of being crowded together. There is no opportunity for the formation of a peribranchial chamber nor even for a ventro-medial union of the two hyoid arches to form the plica opercularis, so characteristic of the Sauropsida as a whole. Consquently each hyoid arch is pulled back on the side of the trunk and there undergoes a further development by itself, persisting long after the other arches have lost their identity. Just before these disappear (Eutaenia sirtalis, H. E. C., Ser. 1349; 7.6 mm.; and E. radix, Ser. 1350 7.4 mm.) the epithelium of the operculum (in the first case from the under side, in the second from the outer side) gives rise to a tuft of cells comparable in point of time and position with the filaments of the chick. Again, however, these are rather small and transitory appearances. To see structures in the reptile, closely comparable to those in the chick it is necessary to examine turtle embryos (*Chrysemys*, H. E. C., Ser. 1078; 10.0 mm.; and Ser. 1083; 11.6 mm.). The first of these (fig. 22) has been placed beside a chick embryo of exactly the same size (fig. 21, H. E. C., Ser. 2038; 10.0 mm.), which happily was so sectioned as to permit a very striking comparison of the two embryos, even to such details as the aortic arches, cephalic veins, etc. A glance at the operculum and its underlying filaments in the two specimens shows that at least in the stage at hand we are dealing with almost identical structures. Again, however, these filaments have but an ephemeral existence as compared with the development which the same structures undergo in the chick.

DISCUSSION OF LITERATURE

Of considerable interest in connection with this paper is the exhaustive work of Ekman on the branchial region of the Anura. He conducted a series of experiments to determine the various factors involved in the production of gill filaments in frogs and

toads. He was able to show by transplantation methods that the ectoderm of the branchial region and immediately adjacent territory has a certain specificity for building gill filaments not possessed by the remaining ectoderm of the embryo; that a polarity of this ectoderm can be demonstrated; and that even when the entoderm and mesoderm underlying the future gill region in very young embryos are removed the ectoderm alone will produce abortive filaments devoid of blood vessels. It is the ectoderm of this same region in reptiles and birds which produces rudimentary filaments and they bear at least a superficial resemblance to some of the abortive structures thus produced experimentally in amphibia by Ekman (cf. figs, 26 and 27). In the case of the higher vertebrates the process never passes beyond the initial stages as evidenced by the early appearance of degeneration vesicles and the failure of blood vessels to participate in gill formation.

In the light of Ekman's experiments and the evidence presented in this paper it is doubtful whether the entodermal invagination which Grosser found in the first pharyngeal pouches of young human embryos has been rightly interpreted as an internal rudimentary gill. Grosser recorded his observations as follows:

A remarkable observation has been made by the author in all young embryos with the first pharyngeal pouches well developed; these are the embryos R. Meyer 335, Hal_2 Pfannenstiel III (loaned for this purpose), R. Meyer 330, and also a somewhat pathological, young embryo from the collection of R. Meyer. In the region of the first pouch there projects ventrally (figs. 315 and 316) or caudally (fig. 318) from the closing membrane into the pharyngeal lumen an irregularly knobbed process filled with mesoderm. That it is an accidental structure or due to post-mortem changes seems to be excluded by the regularity of its occurrence (Low has figured, but not described it). It disappears quite early (in the oldest embryo examined, figure 318) it is present only on the left side and is greatly reduced in size; in embryos of 4.25 5.0 and 5.8 mm. and in those still older, it is wanting), and may perhaps be interpreted as a *rudimentary internal gill.*[4] It would not be the first instance of a very ancient rudiment well developed in the human embryo. Similar structures have not yet been observed in other amniote embryos. (Keibel & Mall Human Embryology, 1912).

[4] Italicized by the author of the present paper.

In his description of embryo "Robert Meyer No. 335," the same author ('11) figures a section of the "gill rudiment" which he describes as follows:

> Das Relief der Gegend der ersten Tasche wird hauptsächlich von der erwähnten, in das Lumen des Vorderdarmes vorragenden Einstülpung beherrscht; sie mag vorläufig als *Kiemenrudiment*[1] bezeichnet werden. Das Kiemenrudiment liegt jederseits ventral und zum Teil kranial von der Berührungsstelle der Epithelien, kaudal vom ersten Aortenbogen und ragt zapfenförmig dorsal und kaudalwärts in das Lumen der Darmbucht vor. In seinem Inneren findet sich ein mesodermaler Kern, Gefässe sind aber in diesem nicht mit vollen Sicherheit nachzuweisen.

There are at least three difficulties in the way of accepting Grosser's interpretation, the first of which involves the entodermal origin of the structure he presents as a gill filament. Kingsley states in his Comparative Morphology, that the gills of vertebrates "were long regarded as of entodermal origin but in recent years considerable doubt has been thrown on this; at least for fishes, and there is some evidence for their ectodermal origin." Ekman has shown that the ectoderm alone can produce abortive filaments in frogs and toads, while the evidence of the present paper establishes the fact that in the Sauropsida the filamentous structures which have been described as vestigial gills are wholly ectodermal. Another factor unfavorable to Grosser's interpretation is the position of this structure in the auditory pouch. If gills have persisted at all in so highly developed an animal as man, it is not likely that they would persist on arches which least commonly possess gills in water-breathing vertebrates. For with the exception of a few cyclostomes and fishes, gills are never found in the hyomandibular cleft. Again, the time of development is against Grosser's interpretation. The inpocketing which he describes first appears at a time when only the first two entodermal pouches have been formed (Embryo R. M. 335, 1.73 mm., 9 to 10 somites) and when the second has not yet reached the ectoderm. It is last met with in embryos no older than R. M. 300 (2.5 mm., 23 somites) where only the first three pouches have reached the ectoderm. In fishes the functional gills are never formed before all the clefts have broken

through, at a considerably older stage than that represented by the human embryos under discussion. Unless, therefore, something similar can be found in the branchial region of the lower vertebrates, it hardly seems as if the structure described by Grosser could be regarded as an internal rudimentary gill. With more likelihood it may be compared with those outpocketings in the preauditory region of the pharynx of the chick which Kastschenko as doubtfully considered "vermutliche rudimentäre Schlundtaschen."

Apparently filaments do not occur in mammals. The extensive series of mammalian embryos which are available in the Harvard Collection have been searched in vain for traces of filaments comparable with those already described for the Sauropsida. Reviewing the phylogeny of the branchial system of vertebrates in the light of these facts, it would seem that the gills of the lowest vertebrates have given place to functionless homologues in the Sauropsida and that with the further reduction which the branchial system has undergone in mammals all traces of even vestigial filaments have disappeared.

CONCLUSIONS

In the Sauropsida the development of the branchial region is characterized by the formation and relatively late persistence of a band of tissue across the ventral surface of the neck, which has been derived from the ventral union of the hyoid arches, and which may be known from its resemblance to the development of the gill cover of certain fishes and amphibians as the opercular fold or plica opercularis (Kiemendeckelwulst of German authors).

On the lateral margins of this operculum, after it has grown backward to enclose at least a potential peribranchial chamber, filamentous outgrowths may be observed on its under side, which in reptiles have a very transitory existence but which in the chick undergo a relatively extensive and prolonged development.

On account of the filamentous character of these outgrowths, their origin from the branchial arches (the epithelium of which Ekman has shown to possess a certain specificity for gill-formation in the Anura), and their constant relation to the operculum

in both reptiles and birds, these structures are adjudged to be true gill filaments, evidently vestigial in character, but none the less comparable in kind to the functional organs of water-breathing anamniotes.

If this interpretation proves to be correct an unbroken series of gill-bearing vertebrates is thus presented from fishes up to mammals.

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PLATE 1

EXPLANATION OF FIGURES

Development of gill filaments in chick embryos of the fourth to the eighth day of incubation

4 Chick; 3 days, 4 hours; 6.8 mm. × 9 diam. (H. E. C., Ser. 2057) *I–IV*, first four ectodermal grooves (cf. with fig. 10).

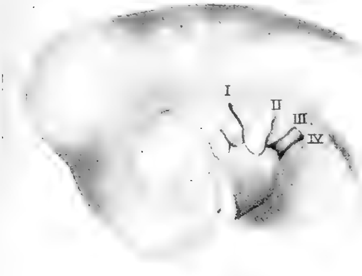
5 Chick; 3 days, 22 hours; 8.1 mm. × 9 diam. (cf. with fig. 11). Note hillock on third arch.

6 Chick; 4 days, 23 hours; 13.4 mm. × 9 diam. I, auditory groove; *II*, *III*, second and third ectodermal grooves limiting the wedge out of which the filaments are differentiating (cf. with figs. 18 and 19).

7 Chick; 6 days, 3 hours; 16.9 mm. × 6 diam. *II*, site of the second ectodermal groove, occupied by a ridge bearing filaments; *S.P.*, pectoral groove.

8 Chick; 6 days, 5 hours; × 6 diam. (cf. with figs. 1 and 20).

9 Chick; 7 days, 1 hour; 19.7 mm. × 6 diam.


I
II
III
IV


II
III

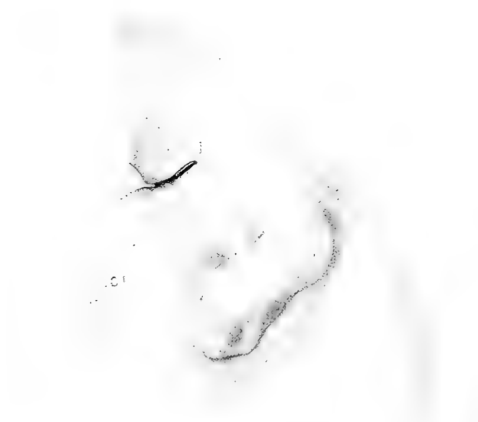


PLATE 2

EXPLANATION OF FIGURES

Sections illustrating early development of filaments

10 Section through the branchial clefts of an embryo of 3 days and 4 hours (H. E. C., Ser. 2057; 6.8 mm.; section 266; × 27 diam.), illustrating the stage when vesicles first appear in the branchial epithelium (cf. with fig. 4). *I–IV* ectodermal grooves; *op.*, operculum.

11 Section of an embryo of 4 days and 4 hours (H. E. C., Ser. 2058; 11.0 mm.; section 482; × 27 diam.), illustrating the stage when vesicles become concentrated in the ectoderm of the third and fourth arches (cf. with fig. 5). Note the thickened epithelium covering the mound on the third arch. *3, 4, 6,* aortic arches.

12 to 17 Consecutive serial sections of an embryo of 4 days and 3 hours (H. E. C., Ser. 1943; 12.0 mm.; sections 215 to 220 respectively, × 27 diam.). The first three, on the left, show the mound which forms on the third arch and its mesodermal core; the last three, on the right, show the thickened epithelium at the lower end of the mound, out of which the first filaments are differentiating.

12 *I, II, III,* ectodermal grooves; *II, III, IV,* pharyngeal diverticula.

15 *E-III, E-IV*, evaginations of the ectodermal epithelium on the third and fourth arches, respectively.

PLATE 3

EXPLANATION OF FIGURES

Sections illustrating later development of filaments

18 and 19 Sections through the hyoid region of an embryo of 5 days; 3.0 mm. (H. E. C., Ser. 1951; sections 245 and 247 respectively) × 67 diam. *3, 4, 6,* aortic arches; *II,* second ectodermal groove; *III,* diverticulum of third pouch; *op.,* operculum; f_1, filaments at the upper end of the 'wedge;' f_2, filaments at the point of the 'wedge' (cf. with fig. 6).

20 Frontal section through the opercular fold and pectoral wall of an embryo of 6 days and 2 hours; 16.0 mm. (H. E. C., Ser. 1950; section 901) × 67 diam. (cf. with fig. 8). *op.,* operculum; *f.,* branched filaments; *s.p.,* pectoral grooves; *c.m.,* mesothelial ridge; *b.c.,* bulbus cordis; *p.,* pericardial cavity; *c.p.,* thickened epithelium which gives rise to the pectoral ridges.

21 Filaments of a 10.0 mm. chick (H. E. C., Ser. 2038; 5 days; section 572) × 67 diam. (cf. with fig. 22). *3, 4, 6* aortic arches; *III, IV,* diverticulum of third and fourth pouch; *e.,* esophagus; *tr.,* trachea; *p.,* pericardial cavity.

22 Filaments of a 10.0 mm. turtle embryo (H. E. C., Ser. 1078 *Chrysemys marginata,* section 286) × 67 diam.

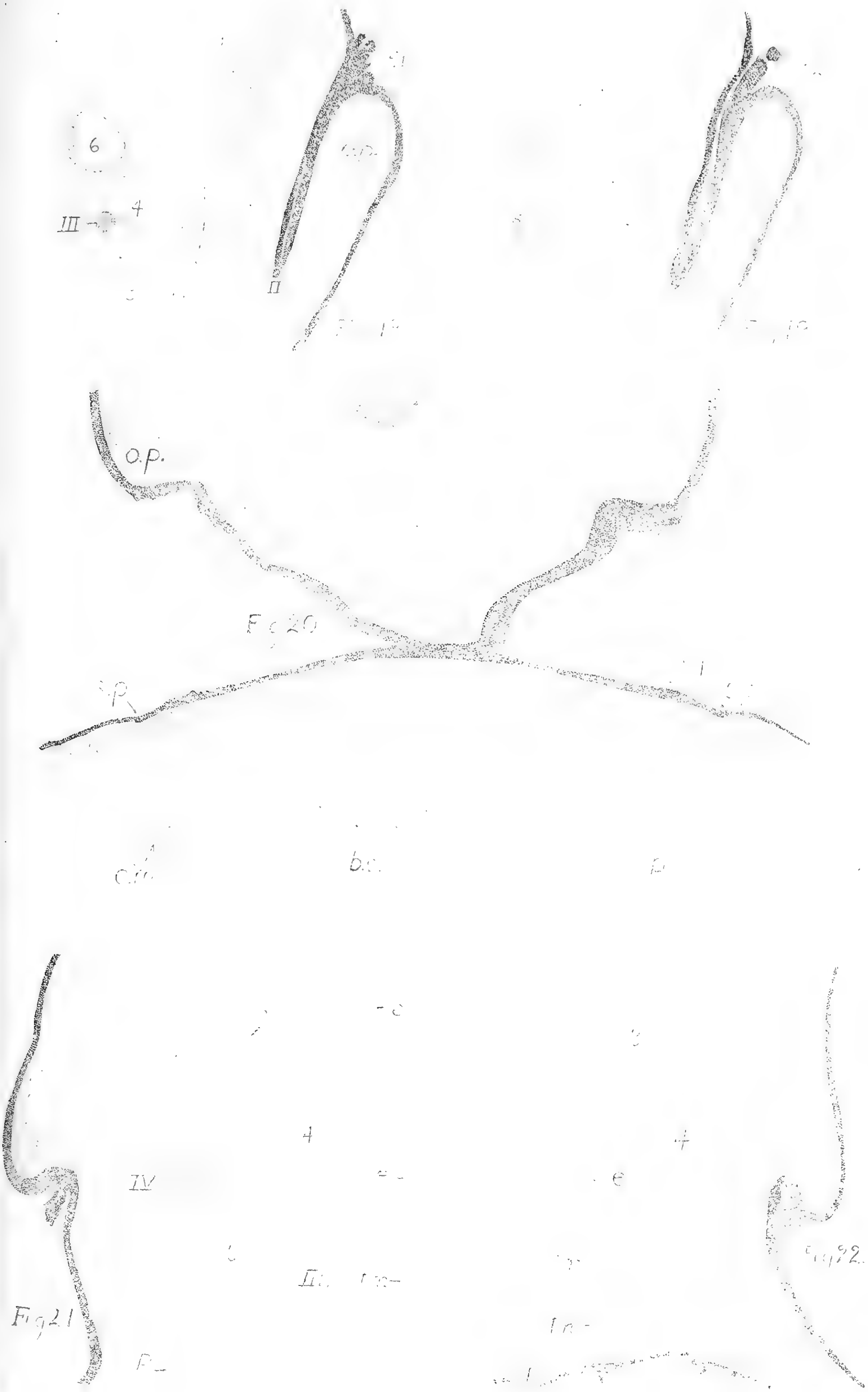

6
III
4
II
o.p.
o.p.
b.c.
IV
4

PLATE 4

EXPLANATION OF FIGURES

23 Low power sketch of operculum and filament from a chick embryo of 4 days and 23 hours (H. E. C., Ser. 2059; 14.0 mm.; section 1004) × 50 diam.

24 High power sketch from same section as figure 23 showing longitudinal section of an opercular filament. Camera lucida drawing × 750 diam. *ect.*, ectoderm covering the under surface of the operculum; *mes.*, underlying mesenchyma.

25 Epithelial cyst in process of formation. Camera lucida drawing of section 207. × 900 diam. (H. E. C., Ser. 1954; 4 days, 3 hours; 9.0 mm.). *ect.*, ectoderm covering the fourth branchial arch; *mes.*, underlying mesenchyma. The vesicle figured measures 19μ in diameter.

26 After Ekman's figure 29: "Horizontalschnitt durch die Kiemengegend einer *Bombinator*—Larve 3 Tage nach der Entfernung der entodermalen Mundhöhlenwand im I. Stadium. *Bg.* Blutgefässe; *KI–III* 1–3. Kiemenreihe; Op., Operculum." × 200 diam.

27 Section through the hyoid region of a 12.0 mm. chick (5 days, 1 hour, × 100 diam.), for comparison between the normally occurring vestigial filaments and opercular fold of the chick and the abortive filaments and peribranchial chamber experimentally produced in toad embryos by Ekman (cf. with fig. 26).

Fig. 26

Fig. 27.

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THE FORMATION AND STRUCTURE OF THE ZONA PELLUCIDA IN THE OVARIAN EGGS OF TURTLES

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TWELVE FIGURES

CONTENTS

INTRODUCTION

In the active research upon eggs of all groups of animals, which has been in progress for nearly fifty years, the zona pellucida, a cuticular membrane, formed around the egg in the course of growth at some stage preceding maturation, has not failed to be an object of interest to investigators and to share with other portions of the egg the most painstaking examination. According to Waldeyer ('01, '02, '03, p. 287) there occurs at least one membrane in all vertebrate eggs whereas among invertebrates some eggs remain naked. The majority of vertebrate eggs which have been subjected to careful study show this membrane to be the zona pellucida. It consists typically of two concentric layers, one of which exhibiting characteristic radiating striations perpendicular to the egg surface is termed the zona radiata.

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That in the same species this membrane varies to a considerable degree in thickness is shown by figures given below where in the turtle's egg its thickness ranges from 1μ in initial stages to 17μ. The variation of this dimension in the eggs of different groups of vertebrates can be demonstrated by comparing these figures with those given by Prenant, Bouin, and Maillard (Traité d'Histologie, p. 1097) who quote Nagel's measurements, 1.2μ to 1.5μ in the mouse and 2.0μ to 2.5μ in man. The zona pellucida must be distinguished from the yolk or egg membrane, a very thin membrane observed in some vertebrate eggs surrounding the yolk before maturation is completed (Van Beneden, '80, Fischer '05, p. 595).

In respect to thoroughness and extent of investigation upon the structure of the zona pellucida, mammalian eggs naturally stand first. Less complete and comprehensive study has been given to it in the lower vertebrate groups.

HISTORICAL SKETCH

Authors who have given detailed contributions upon the mammalian zona pellucida may be grouped into three classes: first, those who regard this membrane as originating from the egg cytoplasm; second, those who maintain that it is derived from processes of the egg epithelium or from an exoplastic or intercellular substance of the cells of this epithelium; third, those who frankly state its origin to be uncertain. The first class includes Van Beneden ('80), Kölliker ('98) and Sobotta ('02). In the normal Graafian follicles of the bat (Rhinolophus ferrum equinum, Schreb.), Van Beneden observed egg cells in such close contact that the zona pellucida of one touched the zona pellucida of its neighbor to the exclusion of the epithelial layers. He, therefore, concluded that the zona pellucida developed from the surface of the egg cytoplasm in the absence of the egg epithelium. Kölliker stated that egg cells not yet surrounded by epithelium but already arranged in islets or nests were inclosed in a distinct membrane which he designated as the first anlage of the zona pellucida.

Flemming ('82), Retzius ('89), Paladino ('90), Von Ebner ('00), and Fischer ('05) affirm that the zona pellucida is formed of cytoplasmic prolongations of the epithelial cells. Flemming describes fibers which "may be protoplasmic connections of the egg cell with its neighboring cells; in the spaces between these bridges the intermediary mass of the zona, gradually becoming firmer, may be laid down." Retzius states that in the rabbit cylindrical cells send out branched processes which gradually interlace so that a thick network originates around the egg. A consolidation occurs on the inner belt of this network forming the zona pellucida. In the completely developed zona pellucida the outer zone is also consolidated and between the inner zone and the surface of the egg radiating striations can be recognized. These represent granular filaments, which bore through the substance of the zona and are attached to the egg surface by small conical basee. In the opinion of Paladino, bridges exist in the rabbit between the epithelial cells as a whole, as well as between these and the egg cell. In ripe eggs a fiber net exists between the epithelium and the outer surface of the egg, the inner meshes of which contain finely granular substance. Paladino gave a rather bizarre interpretation of this granular substance, regarding it as nutrient material derived from the breaking down of the epithelial cells formerly existing in these areas. A true zona pellucida is evolved from this substance which becomes hyalin in character and strongly refractive. Von Ebner substantiates in general the statements of Flemming and Retzius. The first anlage of the zona shows a network closely attached to the egg surface; this gradually moves back toward the epithelium leaving radiating filaments in connection with the egg surface to give place to the "secondary zona substance." According to Fischer the zona pellucida arises from unbranched cytoplasmic prolongations of the epithelial cells interwoven and pressed together. Compression occurs to such an extent on the inner portion of the zona as to eliminate the individual outlines and thus form a homogeneous substance. In the completely developed zona pellucida he distinguishes three layers, spongy, radiating and homogeneous, of which the last named is the oldest and firmest.

Regaud and Dubreuil ('08, p. 152) deny any protoplasmic connections between the egg and the cells of the egg epithelium.

In a fully developed ovarian follicle (rabbit) the zona is formed of three concentric layers. The first is a very thin internal layer applied to the surface of the egg but substantially independent of it; to this layer, which is not homogeneous but fenestrated in the manner of a grating (or grill?), we have given the name fenestrated epiovular membrane. The second is an external layer in connection with the prolongations of the cells of the corna radiata: it is formed by a thick felt of filaments running in all directions, the felted layer. The third or middle layer, the zona pellucida properly called comprises two substances, radiating filaments irregularly extending from the felted layer to the periovular membrane and an amorphous or granular substance (following the action of the fixative), laid down in abundance in the spaces between the radiating filaments which it bathes. . . . The felted filaments, the radiating filaments and the epiovular membrane which have been interpreted up to the present time as anastomosing elements are not protoplasmic but an exoplastic production of the follicular cells about the egg.

The investigations of Rubaschkin and Waldeyer have left them in doubt as to the exact origin of the homogeneous substance (so-called by them) of the zona pellucida. According to Waldeyer ('01, '02, '03) the zona pellucida is composed of a fiber felt and a homogeneous substance across which protoplasmic connections from the epithelium to the ooplasm make their way. The homogeneous substance is perhaps a product of the ooplasm and the mammalian zona pellucida, derived in part from the epithelium, in part from the ooplasm. Rubaschkin ('05, p. 519) describes as the zona pellucida in guinea pigs, a thick homogeneous layer directly surrounding the egg or yolk membrane. Central processes from the epithelial cells penetrate this zona substance where they lose their protoplasmic appearance. These processes do not form intercellular bridges because they are prevented from actual contact with the ooplasm by the presence of the egg membrane. They do not end with enlargements or knobs as Retzius figured them to do. A number of eggs, however, show a thick layer of coarse fibers, the processes of the epithelial cells which wind about the zona substance but do not penetrate it at any point. This layer corresponds to the perizonal fiber net of Retzius. Waldeyer is inclined to regard the zona pellucida, con-

trary to his earlier opinion, as a product of the ooplasm, while Rubaschkin favors the view that it is derived from the epithelium.

Of those who express an opinion on the zona pellucida of the egg in vertebrate groups below the mammals there may be cited Lams on the European smelt (Osmerus eperlanus), Munson on the turtle (Clemmys marmorata), Waldeyer on selachians amphibia, reptiles and birds, and Mlle. Loyez on reptiles in general. In Osmerus eperlanus, Lams ('03, '04) describes the zona pellucida which he calls a chorion, thick and radially striated. The striated appearance is due to innumerable canalicules running perpendicular to the surface of the egg. Also, in the cytoplasm of the egg directly beneath the yolk membrane, he sees granular striations which "do not properly, in all probability, belong to the egg cell but correspond to prolongations of the follicular cells which have traversed the canalicules of the chorion and the yolk membrane and become continuous with the cytoplasm of the egg." Munson ('04, p. 331) states that in Clemmys marmorata there occurs an egg membrane which is composed of two layers, the outer homogeneous and the inner striated. In selachians, amphibians, reptiles and birds Waldeyer ('01, '02, '03, p. 293) shows that a zona pellucida consisting of an outer homogeneous and an inner striated layer can be seen well only in developing eggs, that it atrophies in mature eggs leaving only a very thin egg membrane. The striated appearance is due to radial canals. According to Mlle. Loyez ('05, '06, p. 147) three membranes arise in eggs of reptiles. The vitelline membrane which originates directly from the primitive membrane of the oocyte is at first very thin. As it increases in thickness it becomes finally striated and then granular. The heavily striated zona radiata forms on its inner surface early in the course of development. A very transitory third membrane is differentiated from the internal surface of the zona radiata. After its disappearance the inner surface of the zona radiata becomes less and less distinct and finally the striations come to appear in the superficial layer of the egg. Mlle. Loyez' vitelline membrane and zona radiata together make up the zona pellucida, without doubt and her third transitory membrane is the yolk membrane.

This short résumé proves that most authors agree upon the existence of protoplasmic bridges connecting the epithelial cells with the egg cytoplasm. But when they mention the homogeneous cuticular substance few give satisfactory descriptions and illustrations of the origin of this or of its structure in later phases of development. Only Regaud and Dubreuil go into the subject in detail; they lay emphasis upon the different stages of its development from the exoplastic fibers formed between the epithelial cells. The object of the present paper is to show that this membrane in the species studied consists neither of real cytoplasmic structures nor of real exoplastic structures but of intercellular substance and of cytoplasmic prolongations of the epithelial cells combined in a definite manner. The intercellular substance is represented by a series of walls ramified and anastomosed in such a way as to create cylinders or canals of which the transverse section appears as a reticular network. Extending down through these cylinders cytoplasmic filaments from the epithelial cells make their way to the yolk substance.

MATERIAL AND METHODS (INCLUDING POSSIBLE SOURCES OF ERROR)

Twenty-one series have been prepared from the ovarian eggs of the following turtles: Clemmys guttatus (Schneider), Graptemys geographicus (Lesueur), Emydoidea blandingi (Holbr.), Aromochelys odoratus (Latr.) and Chrysemys picta (Hermann) in various stages of growth. The identification of these species is so simple that I shall not stay to discuss the particular features by which they were identified. The animals were killed as soon as possible after their arrival in the laboratory to reduce errors in observation, the result of any prolonged starvation due to improper feeding, a condition which has marked influence upon the general ovarian structure as shown recently by Walsh (Loeb '17). The time which elapsed between the capture of the turtles and their arrival in the laboratory and the conditions under which they were kept prior to their arrival are not known. The majority of the ovaries examined had the appearance of being perfectly

normal. In a few of them, however, at least one egg which must already have attained a diameter of 2 to 3 mm. showed processes of degeneration well under way. In several of these pathological eggs I found an object which Dr. Van der Stricht and Dr. Todd identified as a parasite. To the influence of this parasite the pathological condition of the egg was probably due but no one to my knowledge has so far made a study of this subject.

All the eggs examined were very much less than the size of the deposited egg. No essential differences are apparent in the structure of the zona pellucida of the various species, hence the stages described below have been chosen as representative of all the material.

The following methods of technique were employed. Fixation by the fluids of Hermann, Flemming or Benda, followed by staining with iron haematoxylin and Congo red or with safranin and picric acid. Fixation by Bouin's mixture or trichloracetic acid followed by staining either with iron haematoxylin and Congo red or with Mallory's connective tissue stain. The sections are cut four or five micra thick. All investigators have studied their material in cross section but, judging from their text and illustrations few have seen the importance of examining tangential and oblique sections. Fischer mentions that he could see the fiber work of the spongy layer very beautifully in tangential sections. In tangential sections Lams is able to interpret the structure of the chorion of Osmerus eperlanus. Dr. Van der Stricht called my attention to the significance of this method of study.

Because of shrinkage in paraffin and because of flattening from the action of fixatives and from the pressure of the knife in cutting the circumference of the egg almost always becomes ovoid: this necessitates the taking of averages from measurements of the long and short axes. Because also of the method of measuring with the camera lucida, the figures given for the diameters of the egg, taken through the zona pellucida, are only approximate. The figures given for the thickness of the zona are more exact, having been obtained from prints of microphotographs by computing the magnification.

The microphotographs, all of which were taken at a magnification of 750 diameters, represent the structure of the zona pellucida in eggs ranging in diameter from 0.65 mm. to 2.6 mm. and from younger stages in which the zona pellucida measures only 1μ up to a stage where it is 17μ in thickness. I do not know if this last measurement may approximate the maximum thickness of the zona since I have no measurements from larger eggs. Mlle. Loyez states that in reptiles the zona is very thin upon completion of development. Since it is extremely difficult in microphotography to focus upon an entire field unless that field is perfectly flat in all its parts some portions of the figures are not sharply defined. The endeavor has always been to focus upon the most important part of the section.

OBSERVATIONS

The epithelium

When the oocyte has reached the size two or three times, at a rough estimate, that of the oogonium from which it originated it is surrounded by a flattened epithelium which remains of one layer throughout the course of development. With the gradual growth of the oocyte the epithelial cells take on a definite prismatic shape and increase in height in the axis perpendicular to the surface of the egg until this axis may become as long as the transverse. The transverse axis appears the longer, however, in the majority of cases especially in the later stages herein described. Upon cross sections through the epithelial layer of oocytes less than 1 mm. in diameter the nuclei of the epithelial cells are seen to be rather widely spaced (fig. 2, *ep.*) while in older stages, because of reduction in size of the nuclei and in content of the cytoplasm, the arrangement is more compact (figs. 3, 7, 9, 10, 11, *ep.*). Occasional mitoses prove that to accommodate the increasing volume of the egg the epithelium extends itself by divisions of its constituent cells. In eggs much larger than those figured very numerous mitoses occur. The epithelial cells are sharply marked off from one another by intercellular channels filled with intercellular substance. Unfortunately this does not

show clearly in the photographs. Some preparations fixed in Bouin and stained by Mallory's connective tissue method, show this substance very clearly colored by aniline blue. The intercellular substance early undergoes a change of constitution and becomes transformed, at the level of the surface of the cells, into the special cement known as the terminal bars (Schäfer '12, p. 86, Stöhr '98, p. 68). It is well known that sections cut perpendicular to the plane of the surface of the epithelial cells show in well fixed and stained preparations a continuous dark line representing the lateral surfaces of the terminal bars sometimes thickened noticeably at points marking the limits of two adjacent cells. In other portions of the sections this line may not be seen but cross sections of the bars appear as dark round spots. The former picture is represented in the turtle's eggs in figure 2, *t.b.* The lateral surfaces of the terminal bars of adjacent cells form a rather thick distinct boundary line between themselves and the oocyte thus marking the beginning of the zona pelucida.

Cytoplasmic bridges of various sorts connect the cells with one another (Fischer '05, Paladino '90). Filamentous and thin or short and coarse, they traverse the intercellular spaces and retain their identity for considerable distances within the cell cytoplasm where they finally mingle with the denser portions encircling the large nuclei (fig. 1 *l.b.*, *s.b.*). A dense opaque mass, the attraction sphere, is closely attached to each nucleus usually either on that face which is nearest the surface of the cell or at one side (figs. 2, 4, *a.s.*). Often such clearness is obtained through successful fixation or through the thinness of the section as to determine the character of the sphere. It is composed of three elements, a small granule (or sometimes two) the central corpuscle in the center or slightly to the side of an oval or circular clear field, the medullary layer marked off from the mass by a distinctly larger, more opaque zone, the cortical layer (Van Beneden). Loosely interwoven filaments extend out from the dense attraction sphere to the clear exoplasm at the periphery of the cells thus forming a delicate network.

Zona pellucida

Solely for purposes of clearness the developmental history of the zona pellucida may be presented in three successive stages.

The first stage covers those phases of formation in which the zona pellucida, on cross section, is but a thin one layered cuticle while on oblique and tangential sections the beginnings of a reticular network are found.

The second stage includes that period during which the zona pellucida becomes divided into two concentric layers, the inner thin and radially striated, the outer, denser with striations more or less obscured.

The third stage is co-extensive with the period of growth during which both layers just mentioned become very much thicker.

Stage 1. The terminal bars, as viewed on a cross section, divide the epithelial cells from the oocyte by an apparently continuous line which at first is thin and uniform but later becomes thicker until it is a cuticle of double contour and of rather uneven outline especially on its deep surface where it lies in connection with the epithelial cells. On this front the junctions of the intercellular substance, separating the lateral surfaces of the epithelial cells, make with the bars triangular thickenings. The change in the terminal bars initiates the development of the zona pellucida. From the time when the cuticle reaches an average thickness of 1μ it may be termed the zona pellucida (fig. 2, *i.z.p.*). Filaments of the cytoplasmic network extending from the attraction spheres (*a.s.*) seem to attach themselves directly to the deeper limit of this cuticle (fig. 2) the actual structure of which is not demonstrable on cross sections. Oblique and tangential sections, however, make it clear that the zona pellucida is of complicated organization even at this early stage. It is perhaps well to explain at once that in an oblique or tangential section of an egg one may see two, three or more irregular rows of epithelial cells, the number depending upon the size of the egg and therefore upon the curve of the epithelial layer. These represent cross sections of the epithelial cells at various heights. These portions in the section furthest away from the yolk show the bases of the cells;

then appear successively the clear cytoplasm and perhaps the basal segments of the nuclei; next various segments through the nuclei; and nearer the yolk, sections through the central spheres and terminal bars and therefore through the incipient zona pellucida. These tangential sections (figs. 3, 4, 5) prove that the cuticle is composed of large polygonal fields (*p.f.*) marked off from one another by a system of dark lines, the terminal bars (*t.b.*). These large polygonal fields are not homogeneous but inclose smaller fields of similar outline formed by a fine pale network, the meshes of which are a little thicker and darker at some points and in close connection with the terminal bars, thus giving the impression of extensions of the bars over the surface of the epithelial cells. The meshes of this fine reticulum seem exactly to overlie the deeper cytoplasmic network (fig. 4 *c.n*) of the cell which arises from the interwoven filaments extending from the central spheres. The zona pellucida then takes its origin as a veil-like formation consisting of a mosaic of terminal bars and polygonal fields within which may be recognized the small, pale areas, future canals of the adult membrane separated by pale and dark filaments giving origin to the future fundamental substance of the adult membrane.

In older oocytes several changes take place. Those portions of the network, in which the meshes are a little thicker and are stained in the same way as the terminal bars, have become much more numerous (figs. 5, 6).

It may render the description clearer at this point to distinguish the network of darkly stained meshes which follows the pattern of the original terminal bars around the large polygonal fields, calling this the primary network (*p.n.*) from that which follows the outlines of the original cytoplasmic reticulum, using for this the term secondary network (*s.n.*) Dr. Van der Stricht observes a similar distinction in structures of the membrana tectoria. The meshes of the primary network appear to send out short extensions to the secondary network and to soften their sharp angles so that these assume circular or oval shapes rather than clear cut polygons (figs. 5, 6, *p.n.*). So far I have been unable to assure myself definitely of a longitudinal splitting of

these meshes and of the development of cuticular bridges connecting the parts as has been shown to take place in the membrana olfactoria (Van der Stricht) although certain figures do suggest such an interpretation. A superficial and older portion of the veil of the zona pellucida shows the beginnings of the adult structure, regular small round spaces inclosing dark granules, the cross sections of prolongations of the epithelial cells (figs 5, *pr.*).

Stage 2. In more advanced phases of growth the nuclei of the epithelial cells are crowded nearer to one another and lie closely on the zona pellucida. A cross section (fig. 7) shows that the zona pellucida has become thicker and is divided concentrically into two layers, the outer of which (*o.l.*) is more or less homogeneous and very dark in the figure whereas the inner (*i.l.*) is less opaque and distinctly striated in a direction perpendicular to the surface of the egg. This layer is separated from the yolk substance by a sharp boundary, the nature of which together with the two layers of the zona must be investigated in tangential sections. The real importance of the study of tangential sections is well demonstrated here for the extremely intricate structure of the zona pellucida, of which one could obtain no true conception from cross sections, is revealed with remarkable clearness. In many preparations, as portrayed in figure 8, *o.l.* the outer denser layer appears separated into three concentric belts, a middle clearer stratum (*s'.*) between two bordering darker thicker strata (*s.s''.*). In other preparations stained either more deeply or very slightly this concentric division into belts is not seen. A completely satisfactory explanation for this phenomenon cannot be given. There is a possibility that it may be due to accidental causes, for instance uneven penetration of the fixative or other media used though its explanation is more probably to be found in differences in constitution between the older and the more recently formed parts of the zona pellucida.

Far from being homogeneous the outer layer consists of clear spaces, the cross sections of a system of canals (*c.*) within which are seen filaments, the cytoplasmic prolongations (*pr.*) from epithelial cells. The canals are separated by a meshwork much thicker and larger than in earlier stages, representing the cutic-

ular part of the zona pellucida (*f.s.*) already observed in the first stage. Immediately beneath the epithelium in the zona (*s.*) a series of polygonal or circular fields occupies an area corresponding to that originally marked off by the primary network. On the whole one receives the impression that merging occurs between the primary and the secondary networks so that distinction between them is no longer possible. The three elements of which the outer layer is composed also make up the clear inner striated layer though in the latter region the network of the fundamental substance of the zona stains far less deeply and appears to be of a much less dense character. The striations (fig. 7, *f.s.*) are undoubtedly produced by filaments connecting the epithelial cells with the yolk and by walls of the tubes of the fundamental substance of the zona which these filaments traverse. Since tubes, canals and filaments occur in the outer layer it seems at first remarkable that the striations in it are not obvious in cross sections. In favorable and largely decolorized preparations, the outer layer does appear striated but in more darkly stained preparations the fundamental substance obscures the prolongations because of its great affinity for the stain. The striation in the inner layer is quite evident in cross sections because its fundamental substance takes up very little stain. The inner layer is evidently the older part of the zona and must have been originally identical in substance with the outer layer, the later differentiation resulting from a change in properties of the older fundamental substance causing it to become less dense and to have less affinity for stains. For the site of active proliferation of the fundamental substance is the surface of the epithelial layer which moves back as the epithelial cells withdraw in the centrifugal growth of the egg. It is a still more significant fact, I believe, that living eggs show striations in the outer layer also: at least I have lately observed this appearance in preparations of more advanced stages of the living eggs of Aromochelys odoratus, the eggs of which differ in no essential manner from those of the species previously mentioned in the general structure of the zona pellucida. In eggs of A. odoratus approximately 1.5 to 2 mm. in diameter examined in normal saline the striations of the

outer layer seemed continuous with those of the inner layer yet the line of demarcation between the layers was in no way obliterated. The difference in the nature of the layers apparently is one simply of refraction since there is no distinct structure dividing them nor indeed any distinguishable boundary line. The presence of an egg or yolk membrane which might have been represented by a sharp line of demarcation between the striated layer and the oocyte in figure 7 cannot be confirmed in tangential sections. The boundary line (fig. 7) seems to be produced by thickenings of the ends of cell prolongations at the points where they reach the yolk. No trace of an egg membrane can be discovered in the living oocytes of A. odoratus.

Stage 3. The inner layer of the zona pellucida grows in thickness comparatively slowly whereas the outer, increasing more rapidly, becomes two or three times as thick as the former (figs. 9 and 10). The area of proliferation often stains very deeply (fig. 9, *a.p.*) so that a densely colored belt borders the surface of the outer layer remote from the yolk. At certain points in the outer layer (fig. 9) are seen cross sections of the canals (*c.*) with their contents (*pr.*) at other points a real striation, the result of rows of granular filaments in continuity with identical rows of filaments in the striated layer. This confirms the observation made on living eggs in which was noted the presence of striation in the outer layer. When the area of proliferation has chanced to stain less deeply one can see that the filaments are actually prolongations extending down from the scanty cytoplasm surrounding the epithelial nuclei into the substance of the zona (figs. 10, 11, *pr.*) There are no indications that these filaments branch as Retzius has reported in the case of the rabbit oocyte but the small conical or knob-like bases described by him appear (figs. 9, 10, *k.e.*) as enlargements of the prolongations. Among the granular filaments within the striated layer there appear more homogeneous elements in continuity with the meshes of the fundamental substance of the outer layer (figs. 9, 10, 12, *f.s.*). In this stage the constituents of the zona are shown to be the same as in stage 2: a system of clear openings, cross sections of cylinders (*c.*) with their contents the prolongations (*pr.*) of the epithelial

cells and the mesh work of the cuticular fundamental substance (*f.s.*, fig. 12, *o.l.*, *i.l.*). In the inner layer the meshes of the fundamental substance stand out more clearly than in figure 8 since they are more deeply stained. Here the tubes and filaments have increased greatly in length and the fundamental substance in amount. In the series of stages showing these elements in various phases of development it can be noticed that whereas in numerous openings the prolongations are very well seen in other openings no contents are perceptible. The absence of prolongations from some spaces may be due first to imperfect fixation and staining, secondly to the real lack of systems of cavities corresponding to and overlying the intercellular spaces and consequently the primitive terminal bars in the first stages of development. A very thin discontinuous line between the knob-like enlargements of the ends of the granular filaments and the yolk substance in figure 9 may represent a real egg membrane. This appearance is very rare and further investigation with more refined methods is required to explain it. In tangential sections one sees nothing convincing of the presence of an egg membrane. It may be as Van Beneden asserts regarding the eggs of the rabbit that it can never be isolated in ovarian eggs until a short time before impregnation. In that case it could not be seen in turtles, eggs as small as these which are at present being investigated.

SUMMARY

1. The epithelium surrounding the ovarian egg in all turtles herein reported is represented by one layer of prismatic cells between the sides of which short and long bridges extend. The intercellular spaces at the surface of these cells are closed by a special cement, the terminal bars. The cell is formed by a nucleus and by cytoplasm consisting of an attraction sphere composed of a central corpuscle, a medullary and a cortical layer. These spheres form a dense endoplasm around the nucleus from which filaments extend to a clear layer near the periphery, the exoplasm in a delicate network.

2. The zona pellucida varies in thickness from 1μ to 17μ according to the stage of development of the egg. Beginning with a stage where it is on an average 3μ thick two different layers appear, the outer denser and thicker and the inner narrower, clearer and striated. In the course of development the outer layer differentiates, grows and extends to a greater degree than the inner.

3. The zona pellucida during its growth is always formed by two or three different elements:

a. The fundamental homogeneous substance filling up the spaces between

b. A system of numerous canals or tubules which inclose

c. Filaments or prolongations of the epithelial cells which are connected with the surface of the yolk. The fundamental substance of the zona pellucida is more abundant and dense in the outer layer than in the inner.

4. The fundamental substance of the zona pellucida is developed as a cuticular element by the terminal bars or primary network, that is by a definite special intercellular cement possessing the property of extension over the free surface of the epithelial cells and forming connections there with the delicate secondary network apparently produced directly by the superficial cytoplasm of the epithelial cells. The secondary network seems able to give rise at its surface to a cement similar to that resulting from the activity of the terminal bars. This superficial cuticular network gradually becomes thicker and by the development of fresh cuticular material builds up the entire fundamental substance of the zona pellucida. The prolongations of the epithelial cells, at first short, traverse the zona pellucida and become longer as this increases in thickness. Enclosed in canals, the prolongations reach the surface of the yolk to end in knob-like enlargements.

5. The structure of the zona pellucida just described presents a condition most favorable for the conveyance of nutritive material from the epithelial area in contact with the maternal capillaries to the actively growing and extending yolk.

In conclusion I wish to acknowledge my indebtedness for constant advice and criticism to Dr. Van der Stricht under whose direction this work has been carried out and to Dr. Todd who obtained and identified the material.

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PLATE 1

EXPLANATION OF FIGURES

For abbreviations see page 256

Fig. 1 Tangential section of the epithelium of an egg 0.75 mm. in diameter from the ovary of Chrysemys picta. Benda. Safranin and picric acid. 5μ. Note the long filamentous (*l.b.*) and short thick (*s.b.*) cytoplasmic bridges connecting the adjacent cells. The intercellular substance is not stained.

Fig. 2 Transverse section of an egg 0.69 mm. in diameter from the ovary of Chrysemys picta. Bouin. Mallory's stain. 4μ. The epithelial cells one of which shows an attraction sphere (*a.s.*) very well are widely spaced. The terminal bars (*t.b.*) form the anlage of the zona pellucida (*i.z.p.*) which is 1μ in thickness.

Fig. 3 Oblique section of the egg represented in figure 2. The zona pellucida (*z.p.*) is seen to develop from a system of large polygonal fields (*p.f.*) marked off by the terminal bars (*t.b.*).

Fig. 4 Tangential section of the egg represented in figure 2. A number of epithelial cells are cut through their bases, others at various heights through the nucleus, a third group through the attraction sphere, a fourth through the cytoplasmic network and terminal bars at their surfaces. Central corpuscles can be seen in some of the spheres. The polygonal fields (*p.f.*) are sharply outlined by the terminal bars (*t.b.*).

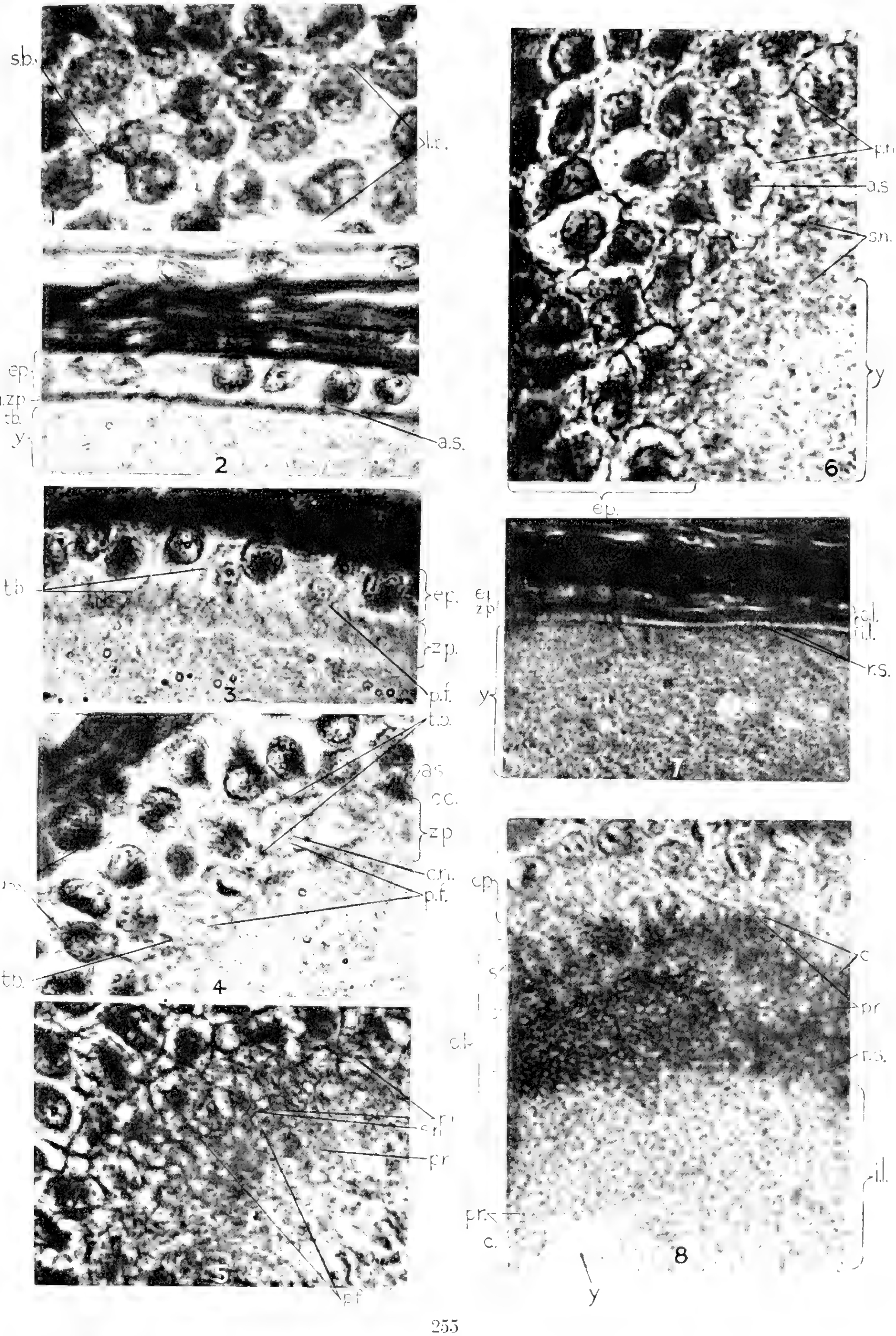
Fig. 5 Tangential section of an egg 0.99 mm. in diameter from the ovary of Chrysemys picta. Bouin. Heidenhain's haematoxylin, Congo red. 4μ. The primary network (*p.n.*) of the zona pellucida follows the outlines of the original terminal bars and the secondary (*s.n.*) the outlines of the superficial cytoplasmic network of the epithelial cells.

Fig. 6 Tangential section of an egg 0.74 mm. in diameter from the ovary of Chrysemys picta. Bouin. Heidenhain's haematoxylin, Congo red. 4μ. The details are similar to those of figures 4 and 5.

Fig. 7 Transverse section of an egg 1.1 mm. in diameter from the ovary of Graptemys geographicus. Trichloracetic acid. Heidenhain's haematoxylin, Congo red. 5μ. The zona pellucida has divided concentrically into two layers, the inner of which shows radiating striations very clearly. It measures 3.6μ in thickness.

Fig. 8 Tangential section of the egg represented in figure 7. Same fixation and stain. 5μ. Both layers of the zona pellucida *i.l.* and *o.l.* are seen to be formed by three elements:

1. A system of canals (*c.*) separated by
2. Meshes of the fundamental substance (*f.s.*) which enclose
3. Prolongations of the epithelial cells (*pr.*)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y

ABBREVIATIONS

a.p., area of proliferation
a.s., attraction sphere
c., canals
c.c., central corpuscle
c.n., cytoplasmic network
ep., epithelium
f.s., fundamental substance
i.l., inner layer
i.z.p., incipient zona pellucida
k.e., knob-like enlargements
l.b., long bridges
o.l., outer layer
p.f., polygonal fields
p.n., primary network
pr., prolongations
r.s., radiating striations
s., outer stratum
s′, middle stratum
s″, inner stratum
s.b., short bridges
s.n., secondary network
t.b., terminal bars
y., yolk
z.p., zona pellucida

The figures are not reduced in reproduction. They are microphotographs taken at a magnification of 750 diameters. Leitz microscope. Obj. 7. Oc. 1.

PLATE 2

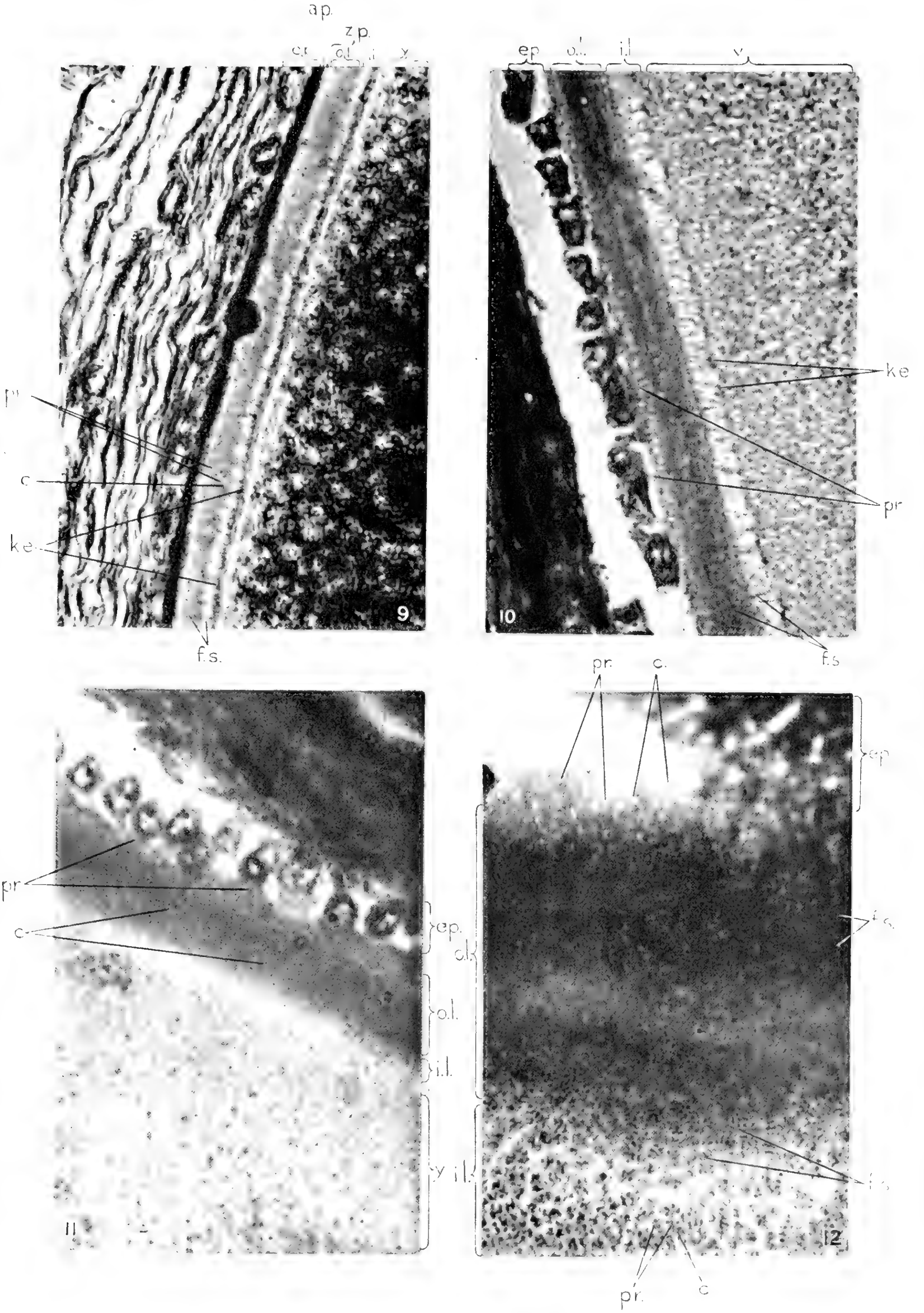
EXPLANATION OF FIGURES

Fig. 9 Transverse section of an egg 1.42 mm. in diameter from the ovary of Clemmys guttatus. Benda. Safranin and picric acid. 5μ. The outer layer (*o.l.*) of the zona has thickened to a greater extent than the inner (*i.l.*). The prolongations show knob-like enlargements at their tips (*k.e.*)*t* The area of proliferation (*a.p.*) is deeply stained. Zone measurement 12μ.

Fig. 10 Transverse section of an egg 2.6 mm. in diameter from the ovary of Chrysemys picta. Bouin. Heidenhain's haematoxylin. Congo red. 4μ. The prolongations (*pr.*) are clearly seen extending from the scant cytoplasm of the epithelial cells down into the outer layer. The zona measures 17μ in thickness.

Fig. 11 Oblique section of the egg represented in figure 10. Same fixation and staining. 4μ. Note the canals and the prolongations of the epithelial cells.

Fig. 12 Tangential section of the egg represented in figures 10 and 11. Bouin. Mallory's stain. 4μ. With figures 10 and 11 this shows the great increase in thickness in both layers of the zona (cf. with figure 8).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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE FONTANELLA METOPICA AND ITS REMNANTS IN AN ADULT SKULL

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FIVE FIGURES

It is not uncommon to find in the skull of a newborn infant a small fontanelle between the two frontalia in their nasal third. This is usually called fontanella metopica (f. medio-frontalis, fonticulus interfrontalis inferior) (fig. 1). A considerable number of skulls, both of children and of adults, showing short, irregular, transverse or V shaped sutures or fissures in the midline of the frontal bone above the level of the superciliary ridges have been described in the literature and interpreted as remnants of the fontanella metopica. The author has found in the skull of an adult an abnormal suture, which is comparable to those above mentioned, but which is more extensive than in any of the cases previously described; accordingly its publication appears justifiable. This specimen (fig. 2) belongs to the Anatomical Department of the Johns Hopkins Medical School and was kindly placed at my disposal by Dr. W. H. Lewis.

The skull is that of an American negro, fifty-five years of age. It might be mentioned that the skin over the frontal region was absolutely normal, therefore any external factor, whether accidental or surgical (trepanation) can be excluded as the cause of the anomaly. The greatest length of the skull is 193 mm., the greatest breadth 148 mm., the basion-bregma height 128 mm. and the horizontal circumference 553 mm. The weight of the skull, including the mandible, is 985 grams, a figure, which is close to the upper limit of variation of weight for the human skull. This is an indication of the thickness of the bones of the skull, which is characteristic of the negro. Most of the sutures are

Fig. 1 Frontal view of the skull of a male negro fetus with a fontanella metopica.

Fig. 2 Frontal view of the skull of a negro with an abnormal suture on the frontal bone.

obliterated, on the inner surface more than on the outer. This is also true of the internasal suture, although its course can still be recognized (the suture was retouched in fig. 2), and therefore the right nasal bone is found at its upper end to extend far into the left. On each side, at the incisura parietalis, there is a Wormian bone. The lambdoid suture is rich in Wormian bones. It is noteworthy that there is present on both sides of the mandible a well pronounced processus anguli mandibulae (apophysis lemurica), which points downward and outward and shows rough outlines for muscle-insertion. The latter are likewise present on the thick zygomatic arch. Attention may also be called at this point to the prominent processus marginalis on the posterior border of the malar bone. The processus anguli mandibulae assumes in our case special interest, in as much as Herpin ('07) reported that this anomaly is rare in the negro and when present is poorly developed.

The abnormal suture on the frontal bone, which is situated not exactly median but somewhat to the right, consists on its outer surface of a transverse, irregular, dentate part, 15 mm. long, and of two lateral, ascending limbs, which diverge upward and have a length of 9 and of 13 mm. on the right and on the left respectively. The distance between the upper ends of these diverging limbs is 23 mm. The middle of the transverse part is situated 25 mm. above the nasion and 15 mm. below the line connecting the two tubera frontalia. If, as according to Schwalbe ('01) the length of the frontal arc is represented as 100, then the transverse portion of the suture lies 20.3 above the nasion. It is of interest to compare the position of the abnormal suture on the frontal bone in the author's case with those reported by Schwalbe ('01), Fischer ('02) and Davida ('14). Table 1 is a compilation of the tables of the two first mentioned authors with the corresponding measurements of Davida's case and of that herein described. The figures show that the suture or fissure is always situated below the level of the tubera frontalia, and with only two exceptions always in the upper half of the nasal third of the nasion-bregma arc. In the twelve European skulls the average relative distance between the nasion and the

TABLE 1

Position of the transverse abnormal suture on the frontal bone of adults

AUTHOR	RACE	AGE	SEX	DISTANCE OF THE SUTURE FROM NASION IN PER CENT OF THE FRONTAL ARC	POSITION OF THE SUTURE BELOW INTERTUBERAL LINE IN MILLIMETERS
Schwalbe	European	32 y.	♂	18.5	17.5
	European	ad.	♂	17.3	11.0
	European	31 y.	♂	17.1	19.0
	European	58 y.	♂	20.3	19.5
	European	41 y.	♂	13.8	20.0
Fischer	European	ad.	♂	18.0	18.0
	European	64 y.	♂	23.9	19.0
	European	40 y.	♂	22.2	20.0
	European	ad.	♂	18.5	16.0
	European	ad.	?	18.2	15.0
	European	41 y.	♀	20.0	29.0
	European	19 y.	♀	17.4	16.0
	Negro	ad.	♂	27.5	12.0
	Negro	ad.	♂	21.8	11.0
Davida	?	50 y.	?	15.4	
Schultz	Negro	55 y.	♂	20.3	15.0

suture is 18.8 mm., and the distance between the intertuberal line and the suture 18.3 mm. The average of the two negro skulls of Fischer and that of the author's case is, for the corresponding measurements, 23.2 and 12.6 mm. respectively. Therefore the suture in the negro seems to be relatively higher above the nasion and closer to the intertuberal line than in the white. The exact determination of the position of this abnormal suture is also of importance in the explanation of its origin, as will be seen later.

Upon examining the inner surface of the skull, the suture is likewise found to be extensive (fig. 3). Incidentally it might be stated that the crista frontalis interna is only moderately developed, as was the case in the skull with the same anomaly described by Rauber ('03). In two of Schwalbe's cases the crista frontalis was examined, and found in both to be broad and blunt. For the most part the abnormal suture on the inner surface communicates with that on the outer surface, often allowing the passage

of a fine bristle. The transverse portion presents itself on the inner surface as eight short perpendicular adjacent fissures, with a total width of 9 mm. and located 19 mm. above the foramen coecum. The lateral limbs of the suture, which also diverge upward, are straight regular fissures, in contrast to those on the tabula externa. The right limb is 13, the left 19 mm. in length and they are 16 mm. apart at their upper ends. The bony part included by the suture is narrower but higher on its inner side than on the outer. On a horizontal section through the frontal

Fig. 3 Frontal bone of the skull in figure 2 seen from the inside (upper part sawed off).

bone, at a level somewhat above the transverse portion of the abnormal suture, a trapezium is formed by the lateral limbs, with its shorter base directed inward. This wedge-shaped piece of bone is plainly shown in Rauber's section of a similar case.

Schwalbe directed attention to the fact that adult skulls showing remnants of a fontanella metopica present an unusually large interorbital breadth. Table 2 is a compilation of tables by Schwalbe and Fischer with Rauber's case and that of the author, in which the interorbital breadths and the interorbital indices are given. The interorbital index represents the relation between the interorbital breadth and the internal biorbital

TABLE 2

Interorbital breadth and interorbital index on adult skulls with the abnormal suture or fissure on the frontal bone

AUTHOR	RACE	SEX	INTERORBITAL BREADTH	INTERORBITAL INDEX
			mm.	
Schwalbe	European	♂	28.5	26.6
	European	♂	32.0	30.2
	European	♂	27.5	27.5
	European	♂	28.0	26.4
	European	♂	31.0	29.8
Fischer	European	♂	31.0	30.1
	European	♂	29.0	26.9
	European	♂	29.0	26.6
	European	♀	24.0	25.8
	European	♀	30.0	29.4
	Negro	♂	26.0	26.3
	Negro	♂	37.0	33.3
Rauber	European	♂	30.5	
Schultz	Negro	♂	32.0	29.1

breadth; the technique of these measurements may be found in Schwalbe's studies on Pithecanthropus erectus ('99). In this same work are published similar measurements of a considerable number of normal skulls of most heterogeneous races. According to these measurements, the interorbital breadth varies between 18 and 31 mm. with an average of 24.2 mm., the interorbital index lies between 20 and 30.1 with an average of 24.3. A comparison of these figures with table 2 shows that both the absolute and relative interorbital breadths of skulls showing remnants of a metopical fontanelle are much above the average.

For the determination of the relative frequency of the anomaly in the two sexes, the material at our disposal has been much enlarged through the published cases of transverse fissures in the frontal bone of children and newborns. In Schwalbe's cases sex is stated in 9 juvenile and in 5 adult; all were male except one newborn. Fischer found in 1 newborn and in 7 adults, in which sex was known, that the female sex was represented twice.

Adding to these the case of Rauber and that of the author, both of which were males, the total of males is 21, of females 3. The anomaly, therefore, would appear to be of much greater frequency in males. This same preponderance has been found by the author ('16) in another anomaly, namely, the persistent canalis cranio pharyngeus, and this relatively greater frequency has been likewise shown in respect to other anomalies. From this it would seem probable that anomalies are more common in the male, but whether this is a rule for progressive or for atavistic anomalies, or for both, can only be determined when care is taken by investigators to always mention the sex in reporting anomalies.

Short transverse sutures or fissures occurring in the lower third of the frontal arc in adults have always been interpreted by the various authors as remnants of the fontanella metopica, but the origin of the latter has been explained in widely different ways. The metopic fontanelle was first described by Gerdy in 1837. He was followed by Hamy and the Italian scientists Maggi, Riccardi, Staderini and Zanotti. Of these, Hamy ('72) sees in the metopical fontanelle a divergence of the lines of ossification of the tubera frontalia. Maggi ('94, '98, '99) interprets the fontanella metopica as a product of the approximation of the four frontalia media. These assumptions are based upon his isolated comparative anatomical observations. Zanotti ('01) explains the medio-frontal fontanelle as the last trace of the foramen, which corresponds to the location of the paraphysis in primitive vertebrates; in other words, a foramen frontale for the paraphysis similar to the foramen parietale for the epiphysis. Both Maggi and Zanotti to a certain extent place atavistic interpretations upon the fontanelle, but these must be considered as extremely hypothetical.

Bolk ('11) was led to believe that the fontanella metopica arises at the site of the primitive or primary nasofrontal suture. This opinion was based upon observations on monkeys, in which the nasal bones have become shortened, that is the supramaxillary portion of the nasalia is displaced by a medial growth of the frontalia, by which process a secondary naso-frontal suture, situated closer to the apertura nasalis, is formed. This theory

does not explain in a satisfactory manner the extremely rare occurrence of a true metopic fontanelle in monkeys, together with the relatively frequent appearance of incomplete nasal reduction. On the other hand, the relative frequency of the metopic fontanelle in man according to Schwalbe is 15.2 per cent in children up to $1\frac{1}{2}$ years, whereas high reaching nasal bones, such as are found in monkeys, have never been described in the human skull. Moreover, it must be borne in mind that the remnants of the fontanella metopica are often situated in the adult high above the nasion. As shown in table 1, the lowest point of the remnants of the fontanelle is located as much as 27.5 per cent of the frontal arc above the nasion, its middle point, being even higher. If the fontanelle really corresponds to the original uppermost end of the nasalia, then the latter must have extended between the frontalia high above the orbits and the superciliary ridges. Bolk assumes that the supranasal portion of the frontal suture (supranasal field or triangle)—a frequent finding in adults—is the result of the reduction of the nasalia. However, this supranasal suture reaches as a rule only slightly above the glabella and not, as Bolk supposes, to the level at which the fontanella metopica occurs.

Rauber ('06) describes the skull of a child with two fontanelles at the frontal suture (fonticulus interfrontalis superior et inferior) which in his opinion had become separated from the frontal arm of the anterior fontanelle. The fonticulus interfrontalis inferior corresponds to the metopic fontanelle, and as a factor in its remaining patent Rauber considers it possible that the site of the anterior neuropore of the medullary canal of vertebrates exerts its influence under special circumstances, even to the ossification of the skull.

Schwalbe ('01) in contrast to the explanations offered by previous authors, considers it possible that the metopic fontanelle is to be conceived as a progressive variation, which bears a relation to the greater development of the frontal lobe of the cerebrum. The adult skull described in this paper would seem to support this theory inasmuch as its capacity was 1520 cc. and its smallest frontal width was 109 mm. Both these measurements are rather large for the negro; on the other hand Fischer's cases

showed the metopic fontanelle to be present in two idiots, one of them a microcephalus with a skull capacity of only 704 cc. Schwalbe in his explanation makes use of the hypothetical supposition that the tubera frontalia might consist of two adjacent ossification centers, which usually join immediately, but in exceptional cases remain separate, later forming two independent systems of lines of ossification. The divergence of these lines forms the metopic fontanelle, which in children is situated on a plane with the tubera frontalia. Schwalbe emphasizes the fact that the metopic fontanelle and its derivatives are always found at a definite location, while the fontanelles and fontanelle bones which are found at times in the upper portion of the frontal suture have a more variable situation and are to be included in the great fontanelle. Schwalbe cites among other the cases described by Staderini, in which the fontanella metopica is connected with the great fontanelle by a wide space. In spite of this, however, he makes a distinction between the two above mentioned fontanelles, which rests purely upon the situation of the metopic fontanelle. According to Schwalbe in children up to 13 months the latter varies in respect to the lower end of the fontanelle from 5.6 to 17.8, in respect to its middle point from 11.2 to 22 per cent of the frontal arc above the nasion. Fischer described the skulls of two children in which interfrontal fontanelle bones are divided in two and in three parts respectively. In one of these the middle point of the fontanelle bone was situated 30.6 in the other 50 per cent of the frontal arc above the nasion.

It is evident that the position of the metopic fontanelle is not as definite as claimed by Schwalbe, who makes the following statement:

> In the rare cases in which two or even three groups of Wormian bones occur in the frontal suture, only the lowest corresponds to the normal medio-frontal fontanelle; those situated near the parietal bones, however, are to be considered as Wormian bones in an abnormally wide suture (hydrocephalus). The latter may even represent the anterior end of the large fontanelle, which has extended abnormally far into the frontal region. It sometimes occurs that the anterior end remains open for a longer period than that portion lying directly posteriorly; therefore the anterior end may become separated as a secondary fontanelle.

This distinction of Schwalbe seems somewhat arbitrary, inasmuch as all transitions can be observed in juvenile skulls. On the basis of original observations the author is convinced that the metopic fontanelle is derived from the bregmatic fontanelle, and at some time has become separated from it. Figure 4 gives the best proof. An interfrontal suture wide at its upper part, that is, a very long arm of the great fontanelle, as shown in numbers 1, 2, 3 and 4 in figure 4 is not of rare occurrence. Among 35 skulls of infants up to a few months' old the frontal arm of the great fontanelle was found to extend six times to within 10 to 17

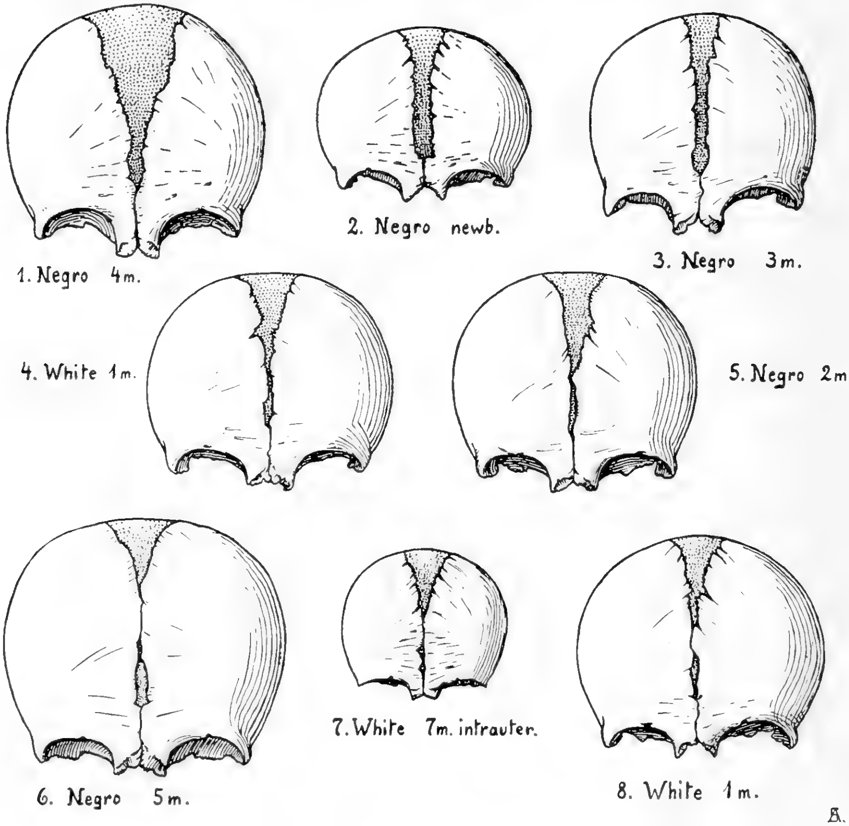

Fig. 4 Normae frontales of frontal bones of juvenile skulls with a long arm of the bregmatic fontanelle, which has been constricted in the lower cases to form a metopic fontanelle.

mm. of the nasion. In three other cases the great fontanelle reached within 22 mm. of the nasion. This prolonged arm of the great fontanelle is an extreme variation, and is not necessarily a result of hydrocephalus. In the skull of a year old hydrocephalic negro, the author found the great fontanelle reaching to within 16 mm. of the nasion; in contrast to the cases in figure 4, however, it was, even at its lower end, 17 mm. wide; in the middle of the frontal arc 29 mm. and at its upper end 35 mm. It is striking that the lowest portions of the frontal bones always approximate

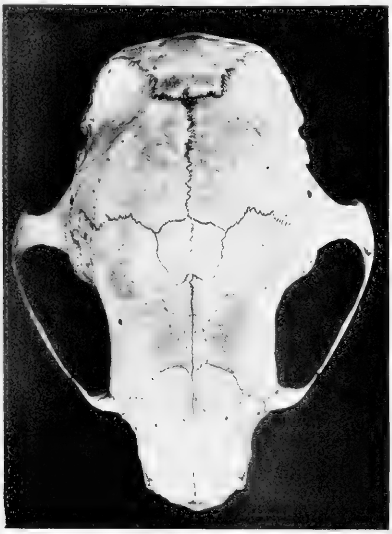

Fig. 5 Norma verticalis of a skull of erethizon dorsatus with fontanelle bones.

each other and indeed to a height which is considered typical for the position of the metopic fontanelle, that is, to a point to which the frontal arm of the great fontanella may extend uninterrupted or constricted. As a designation for the lowest portion of such long bregmatic fontanelles extending into the nasal third of the frontal arc, the name fontanella metopica may well be retained. However, no fundamental difference is to be made between the two mentioned fontanelles. It is more frequent for the lower end alone to remain patent in children and

to be recognizable in adults. The constriction of different portions of the frontal arm of the great fontanelle results from locally decreased or increased growth of the lines of ossification, and may occur in any situation, but appears to be most common between the two tubera frontalia. Double constriction to form secondary fontanelles has also been described (Rauber '06). This identity of the metopic and the great fontanelle is also demonstrated by the position of the fontanelle bones, which occur anywhere from the bregma to the upper portion of the nasal third of the frontal arc (Hartmann 1869, Barclay-Smith '09 and '10, Gulliver 1890). Whether the above described case of partial persistence of the metopic fontanelle in an adult was associated with a fontanelle bone can not be determined with certainty, but seems probable, especially upon examining the inner surface.

Before any definite statements can be made as to the cause of the occurrence and partial persistence of a long frontal arm of the great fontanelle, more material must be available, and attention must be paid to correlations, especially in the frontal region. The author hopes by this contribution to stimulate interest in this anomaly in order that further cases may be reported. Observations on the occurrence of fontanelle structures in the frontal bones of mammals have been reported in a limited number, and further cases would be of great value. Among 10 skulls of erethizon dorsatus, which the author collected recently, 3 cases presented paired symmetrical fontanelle bones extending far between the frontalia. Figure 5 shows one of these cases.

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED BY
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THE ISOLATION, SHAPE, SIZE, AND NUMBER OF THE LOBULES OF THE PIG'S LIVER

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TWELVE FIGURES (TWO PLATES)

INTRODUCTION

The following description of the lobules of the pig's liver is based on a study of lobules that were isolated from one another by means of an acid macerating fluid. This method of isolation is invaluable in giving one a correct idea of the shape and size of the hepatic lobule, and in addition, affords a good means of approximately estimating the total number of lobules in the liver. If the maceration is stopped at just the right point, the method permits the easy dissection of blocks of liver tissue. Dissections of injected livers made in this manner, with the blood vessels and bile ducts as little disturbed as possible, give one a clearer understanding of liver structure than can be obtained by any other method.

A survey of the literature shows that to Wepfer belongs the credit of discovery of the lobule of the liver. In a letter to Paulli (1665) signed by Wepfer, 1664, the substance of the liver was described as follows:

> Examine carefully boiled pig's liver; remove the external membrane and you will find the whole large mass a combination, as it were, of innumerable small glands. Concerning the livers of other animals, I confess, I have not yet made investigations. But upon thoroughly boiling a piece of pig's liver, I have seen small glands, quadrangular and other forms.

In 1666, Malpighi, unaware of Wepfer's discovery, described the lobular nature of the liver in molluscs, the lizard, ferret, mouse, squirrel, ox and man. Concerning those of man, he states:

Finally, in the human body if one will take the care to wash out the blood which is found in the liver by the injection of water, one will observe all the substance of the liver tissue to be composed of a number of small lobes, which resemble, as in other animals, a bunch of grapes.

The lobules were again described by Malpighi in 1683 and in his Opera Posthuma (1698), he accredited Wepfer with the priority of discovery.

A most noteworthy and often cited contribution to the subject of liver lobules is that of Kiernan, 1833. He states:

The form of the liver lobules will be now easily understood: their dimensions are known to all anatomists. They are small bodies arranged in close contact around the sub-lobular-hepatic veins, each presenting two surfaces. One surface of every lobule, which may be called its base, rests upon a sublobular vein, to which it is connected by an intralobular vein running through its center, the base of the lobule thus entering into the formation of a canal in which the sublobular vein is contained. The canal containing the hepatic veins may be called the hepatic-venous canals or surfaces; and as the base of a lobule rests on the sublobular vein, it is evident that the canals containing these veins are formed by the bases of all the lobules of the liver. The external or capsular surface of every lobule is covered by an expansion of Glisson's capsule, by which it is connected to and separated from the contiguous lobules, and in which the branches of the hepatic duct, portal vein and hepatic artery ramify. All the lobules resemble each other in their general form, and they are all of nearly equal dimensions, they appear larger when the section is made in the direction of the hepatic vein, and smaller when in the transverse direction.

Although in few details the above description is incorrect, on the whole it gives one a clear idea of the arrangement of liver lobules. Kiernan's whole paper is full of splendid observations, and one may truthfully say, serves as the basis of our present knowledge of the liver. His figures illustrating the liver lobules, very probably taken from the liver of the pig, have found their way into numerous textbooks of anatomy.

In 1842, Weber called attention to the fact that the lobules of the human liver are not separated from one another as in the pig, and that while lobules are indicated, the parenchyma forms a continuous mass throughout.

The work of Theile, 1884, (cited from Mall, '06) in which are described 'pseudo lobules,' gave rise to a new conception of the

structural arrangement of the liver, although Kiernan in 1833 made the statement that "the essential part of the gland is undoubtedly its duct; vessels it possesses in common with every other organ; and it may be thought that in the above description too much importance is attached to the hepatic veins." We owe to Sabourin ('82, '88) however, the discovery of the true significance of this newly recognized unit of the liver, the unit which is built around the portal canal. This unit, with its imaginary boundaries, has been discussed in recent years by Berdal ('94), Mall ('00 and '06) and Lewis ('04), and has been variously named the biliary lobule, portal lobule, secreting lobule and structural unit by different writers. The value of this latter concept of liver structure is no longer questioned; considered from physiological or morphological view points it stands out as the true unit of the liver. The connective tissue septa dividing the liver into hepatic lobules must be considered secondary both in point of development and importance. Yet in most animals it is the hepatic lobule which appears to be the more definite anatomical structure, and its study is essential to a clear understanding of the portal lobule. With this in mind, and without any intent to emphasize the morphological value of the hepatic lobule, the present study has been made.

THE ISOLATION OF LIVER LOBULES

The method of isolation which I first employed (Johnson, '17), that is, macerating small blocks of formalin fixed liver in 20 per cent nitric acid, I find less satisfactory than the hydrochloric acid macerating fluid used by Huber ('11) in the isolation of kidney tubules. The best method which I have evolved from a number of trials is as follows: Blocks of liver tissue, 1 cm. in thickness, are thoroughly hardened in 10 per cent formalin. They are then placed in 50 to 75 per cent hydrochloric acid and left standing in it at room temperature over night. Next they are placed in an oven (still in the acid) at a temperature of 50° to 60°C. In about two to four hours, depending upon the strength of the acid and the temperature of the oven, the lobules begin to fall apart. The maceration should be stopped when

the lobules separate by gentle shaking. Care should be taken not to allow the maceration to proceed too far, yet it should not be stopped before all the connective tissue is destroyed. The blocks can be tested from time to time by gently pressing them with a dissecting needle. When the maceration is complete, the acid should be diluted four or five times with cold water and the lobules studied in this solution. (When placed in either water or alcohol the lobules disintegrate inside of a day or two.) If dissections of the liver lobules and vessels are desired, such as are shown in figures 11 and 12, maceration should be stopped when the lobules can be torn apart easily with dissecting needles. I have been unable to obtain good results in the isolation of lobules following hardening in either Zenker's or Bouin's fluid or in alcohol, and have been entirely unsuccessful in macerating fresh unfixed liver.

THE SHAPE OF THE LIVER LOBULES

The form of liver lobules is so variable that it is impossible to describe them in terms of any familiar solid. In general, it may be said that they are irregular polyhedrons of a varying number of sides, borders and angles. The surfaces may be plane, convex or concave, and may vary from as few as four or five in some of the smaller lobules to fifteen or more in some of the larger ones. The borders may be either sharply marked or rounded, while the angles formed by the union of the borders may vary from sharply acute to greatly obtuse.

So far as shape alone is concerned I have found no way of determining on which surface the hepatic vein leaves the lobule, the surface which Kiernan ('33) describes as the base. Its point of exit may be either a small or large surface, plane, convex or concave, or it may even proceed from one of the borders or angles of the lobule (figs. 5, 6, 9 and 10).

The surface lobules (figs. 1, 5, 9 and 11) are in many instances distinguishable from the deeper lobules in that they are often irregularly prismatic in shape, their external surfaces are usually slightly convex and the shape of a four, five or six-sided polygon; the sides are plane or only slightly curving and more or less rec-

tangular. The deeper ends of these lobules are usually irregular in shape and quite often larger or smaller than the surface ends. Occasionally are to be seen lobules which are markedly pyramidal in shape, the apices of which may be directed either toward or away from the surface of the liver.

The fact that the lobules of the liver are closely packed solids leads to the question whether or not they resemble any of the regular geometrical solids which fill space. Of such solids, in addition to three, four and six-sided prisms, may be mentioned the tetrahedron, hexahedron, dodekahedron and the tetrakaidekahedron. The surface lobules, as stated above, tend to be prismatic, but I have found but few of the deeper lobules which approach in form any of the above named geometrical solids. Occasionally, however, one may be found which meets the requirements of one of these solids when viewed from one side, but fails when viewed from the other. Several such lobules are shown in figures 1, 2 and 6. If there is any attempt in development to cut the liver up in similarly shaped units, the adult condition does not show it. It should be further pointed out that the lobules in young stages of the pig, amongst them stages in which the lobules are just beginning to be marked off from one another, likewise show but very few regularly-shaped lobules. Among the factors which might tend to break up any uniformity in the shape of the lobules may be mentioned the splitting up of the lobules to form additional ones (Johnson, '17) the unequal growth and size of the various lobules, and the presence of the portal and hepatic canals.

The statement that the lobules of the pig's liver are completely separated from one another by connective tissue septa is prevalent in anatomical literature. While this is true of the majority of lobules, it will not hold for a large number of them. If a block of liver tissue is macerated in hydrochloric acid there will be seen amongst the completely separated lobules a number which cling together in small clumps of from 2 to 6 lobules each, figures 7, 8 and 10. The individual lobules of these clumps cannot be isolated by shaking or gentle teasing, and a definite tearing of the liver parenchyma is necessary in order to divide them.

The clumps, therefore, must be considered as "compound lobules" (Kiernan) and are due to incomplete connective tissue septa. They undoubtedly are the result of the failure of the septa to grow completely across the lobules in the developing liver, at the time when the lobules are dividing to form additional ones. The evidence of incomplete septa can often be seen in ordinary sections of the adult pig's liver.

THE SIZE AND NUMBER OF THE LIVER LOBULES

The size of the lobule of the adult pig's liver is very variable, great differences existing within any individual liver. The smallest lobules may be no larger than 0.5 mm. in diameter; the largest ones may be 2 mm. or over. Assuming that the shapes of the large and small lobules are approximately similar, it is evident that the largest lobules must be as much as 64 times greater by volume than the smallest ones.

The average volume of the liver lobule is dependent to a certain degree upon the size of the liver, thus in small livers the average volume is less than in large ones. This is shown in the accompanying table.

The total number of lobules in the pig's liver is also quite variable. This can be readily observed with the naked eye when examining isolated lobules of different livers of approximately the same weight—in some the majority of lobules are large while in others they are decidedly smaller.

The method of calculating the average size and number of hepatic lobules, which I have found most satisfactory, is as follows: Rectangular blocks of formalin-fixed liver, with dimensions between 1 and 2 cm., were taken from a liver of known weight. Each block was carefully weighed, placed in a separate dish in 50 per cent hydrochloric acid over night, and then in an oven at a temperature of from 50° to 60°C. After about an hour the surface lobules become swollen and each projects slightly from the surface. The surface lobules now being definitely marked off from one another, were counted under a hand-lens, care being taken not to count twice those lobules on the borders and corners of the block. The block was again placed in the oven and

maceraton allowed to proceed until the lobules separated. The lobules were then counted under a hand-lens, a few being taken out at a time with a pipette and removed to a watch glass. In counting, the individual parts of compound lobules were considered as separate lobules; so also were the cut portions of the lobules which came from the cut surfaces of the block. This number was reduced by one-half the number of surface lobules counted, since I assumed that in slicing a piece of liver, the sum of the cut lobules on one side equals the sum of those on the other. Dividing the number of lobules obtained in this way into the weight of the block gives the weight per lobule, and the weight per lobule into the weight of the liver gives the total number of lobules. The average of a number of counts on nine different livers are given in the table below. The average weight per lobule obtained is 2.41 milligrams and the average number of lobules 702,000. The latter number is somewhat higher than that (480,000) obtained by Mall as the average number of lobules in the dog's liver.

TABLE 1

WEIGHT OF LIVER	AVERAGE WEIGHT PER LOBULE[1]	NUMBER OF LOBULES
grams	*mgm.*	
1132	1.95	570,000
1203	1.40	859,000
1418	2.62	541,000
1658	1.95	850,000
1658	2.21	750,000
1786	2.36	757,000
1886	3.99	472,000
1927	2.98	647,000
1942	2.22	874,000
Average	2.41	702,000

[1] The "average weight per lobule" was obtained from calculations based on counts from several blocks taken from each liver.

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PLATE 1

EXPLANATION OF FIGURES

Isolated liver lobules drawn at a magnification of 12.5 diameters. The greatest extremes in sizes are not shown.

1, 5, 9 Surface lobules

1, 2, 6 Geometrical forms.

7, 8, 10 Compound lobules.

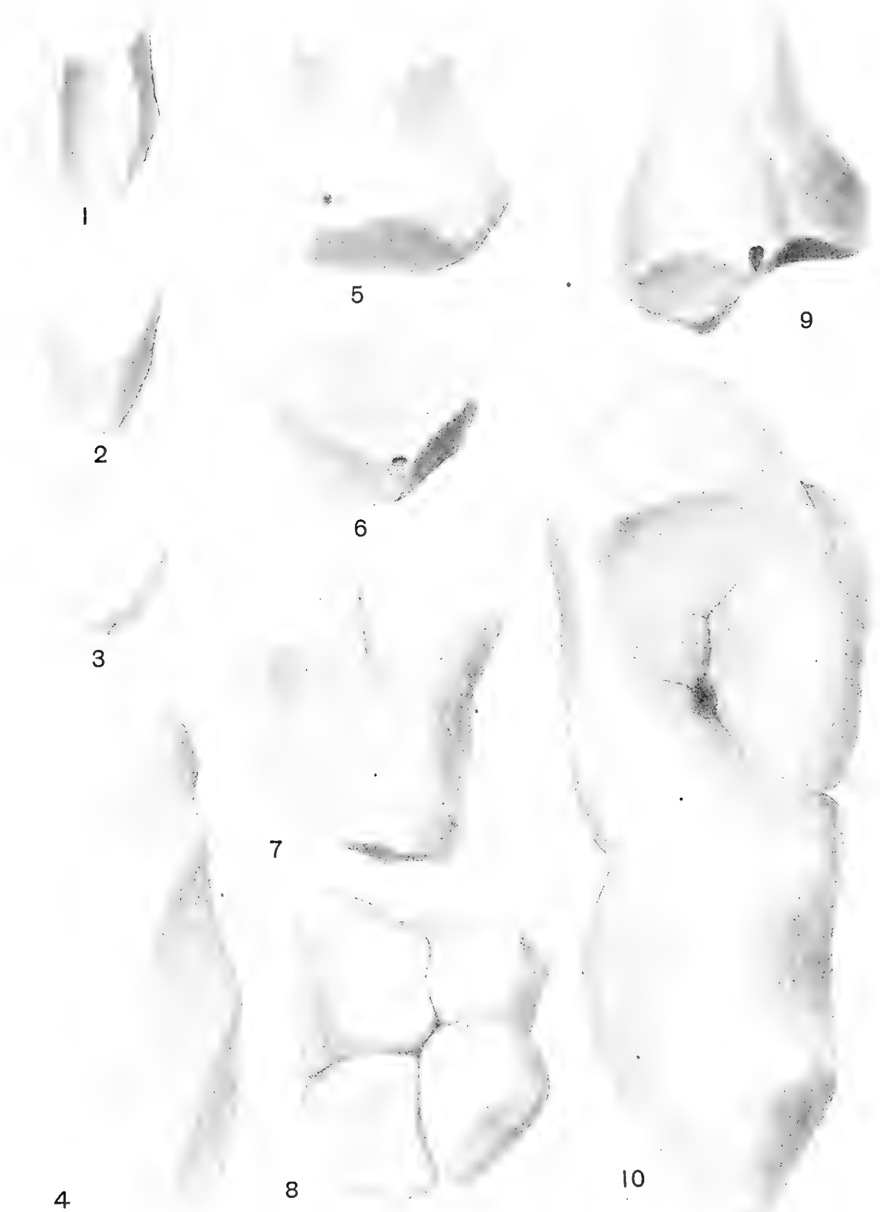
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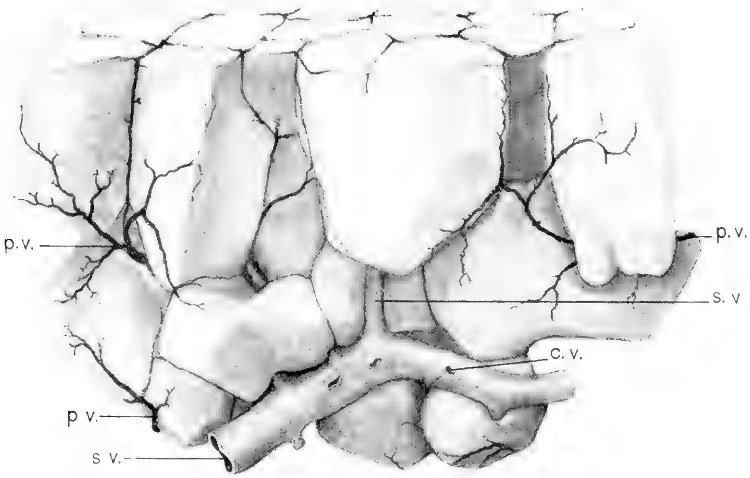
PLATE 2

EXPLANATION OF FIGURES

Dissections of liver lobules to show their arrangement.

11 A group of surface lobules. Bile ducts and branches of the hepatic artery have been omitted.

12 A group of lobules situated deep in the substance of the liver. On the left is seen a large portal canal with bile duct, hepatic artery, and portal vein. The branches of these vessels were worked out as far as possible. Undoubtedly some of them were torn away in lifting off the lobules in dissecting, so that all the branches ramifying over the surfaces of the lobules are not shown. *p.v.*, portal vein; *s.v.*, sublobular (hepatic) vein; *c.v.*, central (hepatic) vein; *b.d.*, bile duct; *h.a.*, hepatic artery.



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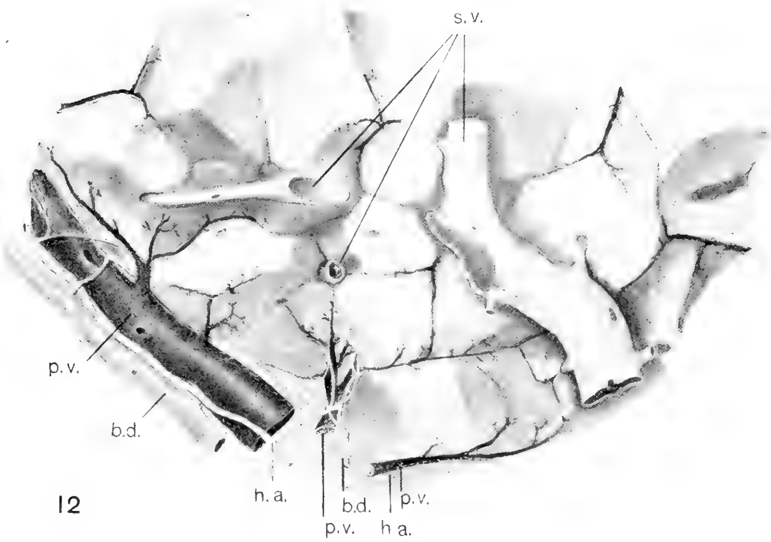

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AUTHOR'S ABSTRACT OF THIS PAPER ISSUED
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THE BRACHIAL PLEXUS OF NERVES IN MAN, THE VARIATIONS IN ITS FORMATION AND BRANCHES

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TWENTY-NINE FIGURES

INTRODUCTION AND METHODS

This paper is based upon records of dissections from the Anatomical Laboratory of the Johns Hopkins Medical School during the years 1895 to 1900, and from the Anatomical Laboratory of the Cornell University Medical College, Ithaca, at intervals from 1900 to 1910.

The dissections were made in most cases by the regular medical students who, after uncovering the peripheral nerves with much care, made records and diagrams of the course, relations, and connections of these nerves.

The dissections were carefully supervised by the instructors as well as by the persons in charge of this investigation. These latter always compared minutely the drawings with the dissection, corrected errors, and wherever necessary made more complete dissections and worked out the finer details. They also recorded the character and accuracy of the dissection and drawing.

The diagrams were compared with the dissections and verified by Dr. A. W. Elting from 1895 to 1897 and by Dr. C. R. Bardeen from 1897 to 1899. The Johns Hopkins records in 1899 to 1900 as well as all the Cornell records were verified by the writer.

From 1895 to 1899, the Elting printed tabular lists of the names of the nerves were used. These were so arranged that the relations and connections of the nerves could be indicated

by underlining, erasing, or inserting the names n the proper place. They were in many cases accompanied by free hand sketches of the arrangement of the nerves and only those records that were accompanied by satisfactory diagrams have been used in this paper. Beginning in 1899, the records were made on the Bardeen Outline Record Charts (Bardeen '00) as prepared under Dr. Bardeen's direction by the writer for the upper extremity. At first the outline record charts were used at Cornell but later the students made natural size drawings.

Between four and five hundred records were preserved. From these I have selected only those diagrams about the accuracy and scientific value of which I could feel no doubt. These number 175. Records have been rejected for various reasons. In some cases, the condition of the dissecting material made it impossible to obtain satisfactory dissections. Lack of manual dexterity or careless dissection made the work of some students of no value. In other cases, the inability of the student to draw accurate or clear diagrams made their records worthless. A considerable number of records which were otherwise accurate were excluded because they did not record the relation of the fourth cervical nerve to the plexus.

It not infrequently happened that the record of the plexus as a whole was satisfactory but the record of one or more of the branches had to be discarded, either because the branch was broken or because, at the time the record was verified, its distribution had not been sufficiently worked out. The record of such a plexus has often been included in this series but has not been used for the study of the doubtful branch or branches. For this reason the number of the records of different branches varies considerably.

Although age would seem to have very little influence upon the course and distribution of the nerves, nevertheless the ages of the cadavers were considered. They were obtained in most cases from the records. In the remainder, they were estimated as accurately as possible. They ranged from infancy to senility.

The color of the subjects used was determined in most cases from the records. Without the records it was often difficult to

distinguish, in the embalmed bodies, light mulattoes from dark whites, and in any case, it was quite impossible to tell whether the colored subjects were pure bloods or were a mixture of negro and white. It is probable that in a great proportion of the cases they were not full blooded negroes. It must be remembered also that the so-called American negroes came from tribes or races of African negroes. In the white bodies, likewise, undoubtedly several and mixed nationalities were represented.

In this paper the number of plexuses and not the number of bodies is given, since not infrequently the dissection or record of one side of the body only was complete or suitable for scientific purposes. In the cases where satisfactory records of both sides were obtained the question of symmetry and asymmetry has been considered in a separate section.

A preliminary statement of the results of the part of this investigation dealing with the formation of the brachial plexus was presented at the 21st session of the American Association of Anatomists in December, 1906, and a synopsis of the findings was published in the American Journal of Anatomy (Kerr '07). A preliminary account of the findings in regard to the subscapular group of nerves was presented at the 23rd session of the American Association of Anatomists in January, 1908, but no record of these statistics has been published.

Much credit for the success of the undertaking is due not only to the students for their careful dissections and records but also to the instructors in charge of the dissection for their interest and cooperation.

I undertook this work at the suggestion of Dr. F. P. Mall, to whom I am greatly indebted for many valuable suggestions. To Dr. C. R. Bardeen I am likewise indebted for much advice and help. I also wish to express my appreciation for the many courtesies extended by Dr. R. G. Harrison. My thanks are due to Drs. Bardeen and Elting for permission to use the records verified by them.

DISTRIBUTION OF THE MATERIAL USED AS TO SEX, COLOR AND SIDE OF THE BODY

It has been possible to divide the plexuses into certain groups and an attempt has been made to determine if one variety of plexus occurs more frequently in white or in colored subjects, in the male or in the female sex, and upon the right or the left side of the body. As the 175 plexuses which were found satisfactory were selected wholly with regard to the accuracy of the record it is at once clear that there would not be an equal number of male and female, white and colored, right and left plexuses. In order to be able to determine the percentage of frequency of each type of plexus among the sexes, the colors, and the sides of the body, it is necessary first to classify the material used.

Table 1[1] shows how irregular this distribution is. It will be seen that 114 of the 175 plexuses or 65.14 per cent are from males, while there are only 61 or 34.85 per cent from females. That is, there are only slightly more than half as many from females as from males.

Of the 114 plexuses from males, 65 are from white and 49 from colored subjects and of the 61 plexuses from females, 20 are from white and 41 from colored subjects. That is, the number from white males is slightly greater than the number from colored males, while there are more than twice as many plexuses from colored as from white females. In spite of this, because of the large proportion of white males, the total number of plexuses from colored subjects (90) is only slightly more than the total number from white subjects (85).

Taking each sex separately or both combined, the plexuses will be seen to be distributed nearly equally on the two sides of the body. This is quite independent of the total number of bodies, since, as already noted, the records from both sides of all of the bodies are not included in the series.

SPINAL NERVES FORMING THE BRACHIAL PLEXUS

All anatomists are agreed that, in man, the anterior rami (ventral primary divisions) of the caudal four cervical nerves

[1] For tables see pp. 376–380.

and a part of the first thoracic nerve[2] always enter into the formation of the brachial plexus. There is more or less vagueness, however, as to the frequency with which one or both of the nerves adjoining these nerves cephalad and caudad also send branches to the plexus. Thus different authors state that there is 'sometimes' a fasciculus from the fourth cervical to join the plexus or that 'frequently,' or 'in many cases' or 'usually' such a branch is present, and similarly that a filament from the second thoracic nerve is found, 'sometimes,' 'frequently,' 'usually,' 'in many cases,' or 'not rarely.' In other words, all are agreed that the anterior rami of at least five spinal nerves enter into the formation of the plexus in all cases, but they are not at all clear as to how frequently there may be six or possibly seven nerves entering the plexus.

In this report, the cephalic limits of the plexus are noted in all instances. The records of the caudal limits are, however, not included, as it was possible to obtain satisfactory records in so few cases that it was thought best to exclude these entirely from the main statistical tables. In some cases the records of the caudal limits of the plexus were not obtained because the second thoracic nerve can only be exposed when the thorax is opened and in many instances this was dissected by a different student than the dissector of the upper extremity. In other cases the nerves were surrounded by strong parietal pleuritic adhesions or were embedded in the shellac mass used for injecting the blood vessels so that it was not possible to make a satisfactory dissection.

Eckhard ('62), Kaufmann ('64), Cunningham ('77), Adolphi ('98) and others have shown that the second thoracic nerve, at times contributes to the brachial plexus. Cunningham found the second thoracic nerve joining the first in 27 out of 37 cases. He says, "Sometimes the connecting twig was very large, sometimes very fine and seen with difficulty. It may be single, double or triple. When double, usually one twig joins the intercostal

[2] For the sake of brevity, the terms fourth cervical nerve, fifth cervical nerve, etc., will be used in most cases instead of anterior ramus of the fourth cervical nerve, etc.

and one the brachial branch of the first thoracic nerve." Harman ('00) found the connection in 7 out of 12 dissections. Paterson ('96) found the connection in only 11 out of 33 cases. Harris ('04) states that it is "only in the postfixed types, in which it might be expected" that the second thoracic nerve joins the first and thus contributes to the plexus. Cunningham ('77) believed that the branch from the second thoracic nerve to join the brachial plexus is influenced by the size of the intercostobrachial nerve. Adolphi ('98) thinks that there is no reciprocal relation between the second thoracic branch to the first and the intercostobrachial nerve. He considers this connection a variation that is associated with a more cephalic or caudal type of plexus and with variations of the thorax and vertebral column. Birmingham ('95) has shown how the communication between the first and second thoracic nerves contributes to the intercostal nerves. The intimate relation and the variability of the connection between the first and second thoracic nerves and the neighboring sympathetic ganglia has been pointed out by Harman ('00).

From the above statement it is clear, I think, that there is much difference of opinion among anatomists concerning the arrangement and connections of the nerves of this region and that they are not well understood and need further study.

DIVISION OF THE PLEXUSES INTO GROUPS

In studying the plexuses it was seen at once that they varied in the number and amount of cervical spinal nerves entering them at their cephalic border.

All those plexuses in which a branch from the fourth cervical nerve enters the plexus fall into one group that has been designated as group 1. The size of this branch varies from a minute twig to a branch as large as the average suprascapular nerve. The group has not been subdivided because of the difference in size of the branch from the fourth nerve. There are 110 of the 175 satisfactory records or 62.85 per cent of the cases that fall in group 1.

There is another group of plexuses in which the whole of the fifth cervical nerve enters the plexus without any additions from the fourth cervical. This type has been called group 2. There are 52 records or 29.71 per cent of the cases in group 2.

There is a third group of plexuses, to be known as group 3, in which not only does no part of the fourth cervical nerve enter the plexus but also the plexus does not receive the whole of the fifth cervical nerve. A portion of the fifth cervical joins with the fourth to aid in the formation of the cervical plexus. There are 13 plexuses or 7.42 per cent of the cases in group 3.

In round numbers we find then that in over 62 per cent of the cases the fourth cervical nerve sends a branch to the brachial plexus and in about 37 per cent it does not. That in this latter case the whole of the fifth cervical nerve enters the plexus in nearly 30 per cent of the cases and only part of the fifth cervical contributes in over 7 per cent.

The three groups into which the plexuses have been divided may be briefly described as follows:

Group 1, in which a part of the fourth cervical nerve enters the plexus, and containing 62.85 per cent of the plexuses (fig. 1[3]);

Group 2, in which the fourth cervical nerve does not enter the plexus but the whole of the fifth cervical nerve does, and containing 29.71 per cent of the plexuses (fig. 2);

Group 3, in which only a part of the fifth cervical nerve joins the plexus, and consisting of only 7.42 per cent of the cases (fig. 3).

Group one

From table 2, it will be observed that 70 of the 110 plexuses in group 1 are from males and 40 of them from females, or 63.63 per cent of the group are from males and 36.36 per cent are from females. There are, however, only 61 plexuses from females while there are 114 from males, table 1. Forty of the 61 plexuses from females are found in group 1, or 65.57 per cent of the female plexuses, table 6. Seventy of the 114 plexuses from males are found in this group, or 61.40 per cent of the male

[3] For figures see pp. 381–395.

plexuses, table 5. We thus see that comparing each sex separately there are over 4 per cent more plexuses from females than from males belonging to group 1.

There are 41 plexuses from white males among the 110 plexuses in group 1 or 37.37 per cent of the group, and 29 plexuses from colored males or 26.36 per cent of the group. There are in all 65 plexuses from white males, and 41 of these or 63.07 per cent are in group 1. Of the 49 plexuses from colored males, 29 or 59.18 per cent are in group 1. It would appear then that among males, the brachial plexus receives a branch from the fourth cervical nerve 3.89 per cent more frequently among the white than among the colored.

Thirty-four of the 110 plexuses in group 1 are from the right side of male subjects, or 30.90 per cent of the group, 36 or 32.72 per cent are from the left side of male subjects, a difference of less than 2 per cent, table 2. Of the 56 right male plexuses studied, 34 or 60.71 per cent are in group 1 and of the 58 left male plexuses studied, 36 or 62.06 per cent are in this group, a difference in favor of the left of 1.35 per cent.

Fifteen, or 13.63 per cent of the 110 plexuses in group 1 are from white females, and 25 or 22.72 per cent of the group are from colored females. There are in all 20 plexuses from white females, and 15 of these, or 75 per cent are in group 1. Twenty-five, or 60.97 per cent of the 41 plexuses from colored females are in group 1. Group 1 plexuses would appear to occur over 14 per cent more often among white than among colored females.

There are 20 right and 20 left plexuses from females that fall in group 1, 18.18 per cent of the group in each case. Of the 31 female plexuses from the right side, 20 or 64.51 per cent are in group 1, and of the 30 female plexuses from the left side, 20 or 66.66 per cent are in the group, a difference in favor of the left of 2.15 per cent.

As regards the two sides of the body, the plexuses in group 1 are distributed nearly equally, 54 on the right side and 56 on the left side, table 2. The total number of plexuses considered is distributed nearly equally between the two sides of the body, 87 on the right side and 88 on the left side. It will be seen then that

62.06 per cent of the plexuses from the right side of the body are in group 1, table 9, and 63.63 per cent of the left plexuses are in this group, table 10, a difference of but 1.57 per cent in favor of the left side.

Of the 110 plexuses in this group, 56 are from white and 54 from colored subjects, or 50.90 and 49.09 per cent of the group respectively, a nearly equal distribution, table 2. But 65.88 per cent of the 85 plexuses from white bodies, and 59.99 per cent of the 90 plexuses from colored bodies are found in group 1. That is, 5.89 per cent more of the plexuses from white than of the plexuses from colored bodies fall in this group.

Forty-one of the 65 white male plexuses or 63.07 per cent of them are in group 1, and 15 of the 20 white female plexuses or 75 per cent of them are in this group, a difference of 11.93 per cent in favor of the white female.

Twenty-nine of the 49 plexuses from colored males are in group 1, or 59.18 per cent, and 25 of the 41 plexuses from colored females are in this group, or 60.97 of them. This gives a difference in favor of the colored females of but 1.79 per cent.

Group two.

From table 3, which shows the distribution of the plexuses from group 2, it will be seen that of the 52 plexuses in the group, 35 are from male and 17 from female bodies, that is, 67.30 per cent from males and 32.69 per cent from females, or more than two to one. Of the total of 114 plexuses from males, 35 fall in group 2 or 30.70 per cent, and of the 61 from females, 17 are in group 2, or 27.86 per cent which shows less than 3 per cent of difference in favor of the males.

Of the 52 plexuses in group 2, 18 are from white males and 17 are from colored males, or 34.61 per cent from whites and 32.69 from colored, table 2. There is a total of 65 plexuses from white males and 18 of them or 27.69 per cent are in group 2. Of the 49 plexuses from colored males, 17 are in this group or 34.69 per cent a difference in favor of the colored males of 7 per cent.

Nineteen of the 52 plexuses in group 2 are from the right side of males and 16 from the left side, or 36.53 per cent right and

30.77 per cent left, a difference in favor of the right of 5.76 per cent, table 6. Of the 56 plexuses from the right side that were studied, 19 are in group 2, or 33.92 per cent, and of the 58 plexuses from the left side, 16 or 27.58 per cent are in this group. This gives a difference of 6.34 per cent in favor of the right side.

There are but 3 plexuses from white females in group 2, as against 14 from colored females. This gives a difference in percentage occurrence of over 21, table 2. Of the 20 plexuses from white females studies, 3 are in group 2, or 15 per cent, while 14 of the 41 plexuses from colored females are in this group, or 34.41 per cent. This gives a difference in favor of the colored of 19.14 per cent.

Nine of the 52 plexuses of group 2 are from the right side of female subjects and 8 from the left side, or 17.30 per cent and 15.38 per cent of the group respectively. Of the 31 plexuses from the right side of female bodies that were studied, 9 or 29.03 per cent are in group 2 and of the 30 from the left side, 8 are in this group, or 26.66 per cent, a difference in favor of the right of 2.37 per cent.

Upon the right side there are 28 plexuses in group 2, that is, 53.84 per cent of the group, and upon the left side there are 24, or 46.15 per cent. There are 28 right plexuses in group 2 out of a total of 87 rights or 32.18 per cent, and out of a total of 88 left plexuses 24 fall into group 2 or 27.27 per cent which shows that in this series, group 2 plexuses are found 4.91 per cent more often on the right side.

About three-fifths of the plexuses in group 2 are from colored and two-fifths from white subjects. Table 3 shows that 21 or 40.38 per cent of the group are from white subjects and 31 or 59.61 per cent are from colored subjects. Twenty-one of the 85 plexuses from white bodies or 24.70 per cent, and 31 out of 90 from colored bodies or 34.44 per cent are in group 2. It would appear then that this group of plexuses is 9.74 per cent more common in colored than in white subjects.

The 21 plexuses from white subjects are 18 from males and 3 from females. Comparing this with the total number from white males, 65, and from white females, 20, we have 27.69 per cent

among the white males and 15 per cent among the white females occurring in group 2, a difference of 12.69 per cent in favor of the white males.

The 31 plexuses of group 2 from colored subjects are 17 from males and 14 from females. Compared with the total number of plexuses from male and female colored subjects, namely 49 and 41, we have 34.69 per cent of group 2 among the colored males and 34.14 per cent among the colored females, a nearly equal distribution between the two sexes among the colored.

From the above, it will be seen that while group 2 occurs among colored males and females about equally often, that it is found very much more often among the white males than among white females.

Group three

Table 4 shows that of the 13 plexuses in this group 9 are from male subjects or 69.23 per cent and that 4 are from females, 30.76 per cent, that is, that more than twice as many are from males as from females. Comparing the number of male and female plexuses in this group with the total number of records from each sex, tables 5 and 6, we find that among the 114 plexuses from males, 9 are in group 3 or 7.89 per cent, and among the 61 plexuses from females, 4 are in group 3 or 6.55 per cent. This shows a variation of but slightly over 1 per cent.

It will be seen from table 4 that 3 of the plexuses, 23.07 per cent, of group 3 are from colored males and 6, 46.14 per cent, are from white males, or exactly 1 to 2. Six of the 65 plexuses from white males or 9.23 per cent are in group 3, and 13 of the 49 plexuses from colored males or 6.12 per cent are in this group, a difference in favor of the white males of only 3.11 per cent.

There are twice as many plexuses in this group from males on the left as on the right side, that is, 6 to 3. Six of the 58 plexuses from the left side of male subjects or 10.34 per cent and only 3 of the 56 from the right side or 5.35 per cent are in group 3, a difference of 4.99 per cent.

In group 3 the plexuses are distributed equally between colored and white females, 2 of each, as the total number of plexuses from

colored females is 41, the 2 in group 3 form 4.87 per cent, while the 2 from white females form 10 per cent of the total of 20 from white females that are in group 3.

Among the females of the group the plexuses are also distributed equally on the right and left sides, 2 on each. Of the 30 plexuses from the left side of the females, 2 or 6.66 per cent are in group 3 and 2 of the 31 or 6.45 per cent are from the right side, which makes the percentage nearly the same on both sides.

Five of the thirteen plexuses of group 3 are on the right and 8 on the left side, that is, 38.46 per cent on the right and 61.53 per cent on the left, a difference of 23.07 per cent in favor of the left side. Five of the 87 right plexuses that were studied or 5.74 per cent and 8 of the 88 left plexuses or 9.09 per cent are in group 3, a difference of 3.35 per cent in favor of the left.

Eight or 61.53 per cent of this group are from white and 5 or 38.46 per cent are from colored subjects. Compared with the total number of records from white and colored, 85 white and 90 colored, tables 7 and 8, we see that 9.41 per cent of the plexuses from white and 5.55 per cent of the plexuses from colored subjects are in group 3, or 3.86 per cent more white than colored.

There are three times as many plexuses from white males as from white females, in this group. The 6 plexuses in this group from white males constitute 9.23 per cent of the total of 65 plexuses from white males while the two plexuses from white females form 10 per cent of the 20 plexuses from white females.

There are 3 plexuses in this group from colored male to 2 from colored female subjects. Of the total of 49 plexuses from colored males, 3 or 6.12 per cent are in group 3 while of the total of 41 from colored females, 2 or 4.87 per cent are in this group, a difference of only 1.25 per cent.

INFLUENCE OF SEX, COLOR, AND SIDE OF THE BODY UPON THE PLEXUSES

The above comparisons of the frequency of occurrence of each group of plexuses among white and colored, male and female, and upon the right and left sides of the body have been made in

two ways: first, within the group, that is, by comparing the number of plexuses in the group from white, colored, male and female, right or left, with the total number of plexuses in the group; secondly, by comparing the number of male plexuses in the group with the total number from males studied and the number from females in the group with the total number from females studied, etc. I consider the latter far the more accurate and shall use that almost entirely in the following comparison of the three groups.

Although comparing the plexuses of each group separately, because of the great preponderance of plexuses from males, the percentage of the plexuses from this sex greatly exceeds those from females, yet if we take the plexuses from males and females of each group and compare them with the total number of plexuses from males and from females it will be seen that groups 2 and 3 occur more often among the males and group 1 among the females. That is, that plexuses in which the fourth cervical nerve enters, plexuses which have the most cephalic origin, occur more often among females than among males. This seems to indicate that the plexus in the female tends to be more cephalic in position than in the male. But we find that the difference in the frequency of occurrence of plexuses from males over females in group 2 is 2.84 per cent but in group 3 is but 1.34 per cent; while if much of any significance were to be given to the greater frequency of group 1 among females we should expect to find them least frequent in group 3, the most caudal group, and not in group 2 intermediate, which happens to be the case.

Furthermore, if we take the plexuses from colored and white males and females of each group and compare them with the total number of colored and white males and females respectively, we find that in group 1 the percentage of colored males is 1.79 less than the percentage of colored females; in group 2, 0.55 per cent more colored males than females; and in group 3, 1.25 per cent more colored males than females. On the other hand the percentage of plexuses from white females exceeds the percentage from white males by 11.93 per cent in group 1; the percentage from white males exceeds the percentage from white

females by 12.69 per cent in group 2, and the percentage from white males exceeds the percentage from white females by .77 per cent in group 3. It must be remembered also that the extreme variation is but a few per cent.

Plexuses of group 2 occur more often on the right side and groups 1 and 3 on the left. Since there is a more or less gradual change from a cephalic to a caudal position in passing from group 1 to group 3 it is difficult to attach any significance to the fact that group 1 occurs 1.57 per cent more often on the left side, group 2, 4.91 per cent more often on the right, and group 3, 3.35 per cent more often on the left.

Whether the form of the plexus is influenced by or influences right and left handedness it was impossible to tell as there was no record as to whether the subjects had been right or left handed. It is altogether improbable that so large a proportion more than half were left handed.

Plexuses of groups 1 and 3 occur more often in white and of group 2 in colored subjects. We have here the same conditions as for the two sides of the body only here the percentage of difference is greater. The difference in percentage in favor of the white is 5.89 per cent in group 1, and 3.86 per cent in group 3; while it is 9.74 per cent in favor of the colored in group 2.

I am quite convinced that so far as this investigation has been carried it does not show that sex, color or side of the body has any influence whatever in determining the group of plexus.

It will be noted that in many of these subdivisions but a small number of cases are considered and that the percentages of variation are in no case great. It would seem not at all improbable that if a greater number of cases were considered equally distributed among the sexes, colors, sides, etc., that the irregularity in this particular would be much less marked.

CEPHALIC AND CAUDAL POSITION OF THE PLEXUS

Various authors have classified the brachial plexus as cephalic and caudal, high and low, or prefixed and postfixed, meaning by this a position or strength of the plexus nearer to or farther from the head end of the body. The classifications are usually based

upon the position of the plexus along the body axis. Those plexuses that receive branches from the fourth cervical nerve would be more cephalic than those that do not. The terms have been used by some, especially prefixed or postfixed, to indicate the position of the supposed greatest strength of the plexus, that is, the position of the largest nerves.

The plexuses of group 1, in which a branch from the fourth cervical nerve joins the plexus may be classified as cephalic, those of group 3 in which the fifth cervical nerve sends a branch to the cervical plexus as caudal, while those of group 2 in which the fifth neither receives nor gives off a branch as intermediate.

Between the most cephalic plexuses of group 1, with the largest sized branch from the fourth cervical nerve and the most caudal plexuses of group 3, with the largest branch from the fifth to the fourth, there is a variation of almost one spinal nerve. This may be accounted for either by a shifting of the plexus along the spinal cord in either a cephalic or a caudal direction without change in its relative size or the number of elements entering it, or by an increase or decrease in the number of nerve fibers entering the plexus.

If it is a shifting of the plexus that takes place, then when the branch of the fourth cervical nerve is large the branch from the second thoracic should be wanting, and when the fifth sends a branch to the fourth there should be a large branch from the second thoracic nerve to the plexus.

If it is an increase or decrease in the number of nerve fibers that enter the plexus that occurs, then the expansion or contraction of the plexus may take place on its cephalic or caudal side or both.

From my own observations just given it is obvious that there is a variation in the cephalic limits of the plexus but unfortunately I have been able to study the caudal limits in only a few of these plexuses. Moreover, I have been able nowhere to find records of cases which show that when the fourth cervical nerve does not enter the plexus the second thoracic invariably does, and inversely, whether when the fourth cervical nerve enters into the formation of the plexus the second thoracic does not, or as to

whether there is a relation in size between these two nerves when they both enter the plexus. The few cases that I have examined seem to indicate an expansion and contraction of the plexus rather than a shifting of it along the cord. I hope that soon we may have sufficient records so as to be able to determine this more definitely.

We must bear in mind that in the spinal cord there are rows or columns of cells extending its whole length from which the nerve fibers take origin or around which they end. The cells are not, so far as we know, arranged in groups corresponding to the groups of fibers in the root fila or in the nerves. The nerve fibers in the case of motor nerves extend from the cells in the gray matter of the cord towards its periphery and then break through the periphery and extend beyond the periphery in groups, the root fila. In the case of the sensory nerves they extend in the opposite direction, that is centrad. The fila radicularia are arranged in continuous rows separated from one another, as a rule, by slight intervals, which are generally somewhat greater between the fila of one nerve and those of the one next cephalad or caudad. As they pass laterad, groups of the fila converge and are joined together into the dorsal and ventral nerve roots. The segmental subdivision of the adult spinal cord in based entirely upon the points of attachment to the cord of the groups of root fila that converge to join a single pair of nerves. Whether or not the same number of nerve fibers enter the same filum in different individuals is not known but it is altogether probable that there is a variation in this respect. We do know that there is a variation in the number of fila which join to form a given ventral root and dorsal root. It is easy to understand how either the appearance of shifting of the plexus along the spinal cord or of its expansion might be produced by variation in the grouping of the fila as they converge to form the nerves, the number of nerve fibers remaining the same.

PREFIXED AND POSTFIXED PLEXUSES. RELATIVE SIZE OF THE NERVES

As already noted some anatomists divide the brachial plexus into prefixed and postfixed groups based upon the position in the

plexus of the strongest elements, that is, the nerves with the greatest diameters.

I have attempted in 27 cases to classify the plexuses in this way, table 11. The anterior rami of the spinal nerves entering the plexus were measured as soon as the surrounding connective tissue was removed. The measurement was made while the plexus was still connected to the spinal cord, that is, before the upper extremity was separated from the body. The diameters were taken with sharp pointed dividers and the measurements were recorded by sticking the points of the dividers into the paper, upon which the diagram of the plexus was made.

Twenty-one of the plexuses are in group 1, and receive a branch from the fourth cervical nerve. This is the smallest branch entering the plexus, it being remembered that the branch from the second thoracic is not considered.

If we disregard the fourth cervical, the smallest nerve entering the plexus is the fifth cervical in 11 instances, and fifth cervical and the first thoracic equally in 9, and the first thoracic in 7. In the 11 cases where the fifth cervical is the smallest, the first thoracic is next in size—the sixth is equally large in 3 of these, the sixth, seventh and eighth in 1 and the seventh in 1. In the 7 instances in which the first thoracic is the smallest nerve, the fifth cervical is the next in size in 5, in two of which the eighth is equally small. The sixth cervical is the nerve of second size in 1 and the eighth in 1.

In the 27 cases, the nerves with the greatest diameter entering the plexus are the seventh cervical nerve in 7 cases, 5 in group 1, 2 in group 3; the eighth cervical nerve in 6 cases, 5 in group 1, 1 in group 3; and the sixth cervical nerve in 2, 1 each in groups 1 and 2. The seventh and eighth cervical nerves are equal in diameter and are the largest nerves in 6 cases, 5 in group 1 and 1 in group 2; the sixth, seventh and eighth cervical are equal and largest in 2 cases both in group 1; the first thoracic, sixth, seventh and eighth cervical nerves are equal and the largest in one instance in group 3; the sixth and seventh cervical nerves are equal and are the largest nerves in 2 cases, both in group 1; and the fifth, sixth and seventh are largest and equal in one instance in group 2.

From the above it is clear that the largest nerve to enter the plexus is the seventh or eighth in 19 of the 27 cases or in over 70 per cent. (The seventh is largest in 7, the eighth in 6 and the seventh and eighth equally large in 6.)

When we attempt to correlate this with the division of the plexuses into groups, we find that in group 1, in which the fourth cervical nerve enters the plexus, the largest nerve and the point of greatest strength is in the sixth cervical nerve in one case, the seventh in 5 cases, the eighth in 5 cases and the seventh and eighth equally in 7 cases. That is, while all 18 of the above plexuses would be classed as cephalic because of receiving a branch from the fourth cervical nerve, those cases in which the largest nerve is the eighth or seventh and eighth would most surely be classed as postfixed and only those cases in which the largest nerve is the sixth and possibly the seventh would be classed as prefixed. That is, each group would have to be subdivided so that the cephalic plexuses (my group 1) would be subdivided into prefixed, postfixed and intermediate.

The diameter of a nerve depends not only upon the number of nerve fibers but also upon the amount of connective tissue, the amount of fat and the quantity of moisture in it. In making a dissection it is very difficult to tell when all the connective tissue has been removed leaving only that tissue which we call epineurium. There is no line of demarcation between the epineurium and the surrounding connective tissue and different dissectors are not liable to agree as to the dividing line. Furthermore in my series it was impossible to tell if a student had removed the same relative amount of connective tissue from the different nerves. The septa that the epineurium sends through the nerve separating and binding together the bundles of nerve fibers are of greatly varying size and blend to some extent with the perineurium that immediately surrounds the nerve bundles.

In very lean persons there is little fat in the epineurium, but in the fat, there is a very considerable amount, especially in the septa between the nerve bundles. An indication of the amount of variation may be seen in sections of a nerve from a fat and a lean body respectively.

In the different nerves of the same plexus, there is also some variation in the amount of fat in the epineurium and of the amount of connective tissue forming the epineurium. The amount of moisture is of less moment though without care it might easily happen that the moisture in the different nerves would be unequal.

It is also extremely difficult to be sure that one is getting correct measurements. Very slight pressure against the muscle dorsal to the nerves such as is caused by traction on the limb or permitting it to drop dorsally will cause a very appreciable amount of flattening. In the comparisons given above, I have endeavored to be very careful to eliminate this source of error so far as possible and have verified each measurement several times, but still do not feel confident of the results. It is, of course, easy to see on inspection that all of the nerves in a plexus are not of the same size, some of them are usually markedly larger or smaller than the others. On the other hand, I feel quite sure that the differences between certain of the nerves especially in the center of the plexus are in many cases so small that under slightly altered conditions two different observers might obtain quite opposite results. Taking all these things into consideration, I feel that the method of classifying plexuses as prefixed and postfixed, based upon the size of the nerves, as we are able to determine them, is of very doubtful value, and that for the human plexus it should have little or no weight as an accessory to other methods.

ARRANGEMENT OF NERVE BUNDLES IN A NERVE

As is well known, each of the anterior rami of a spinal nerve as it passes out between the muscles is composed of a large number of nerve fibers collected into bundles, funiculi. Each funiculus is surrounded by a more or less definite connective tissue sheath, perineurium. These bundles are bound together by more connective tissue epineurium, which also finally forms a sheath around the whole nerve.

The funiculi in a nerve do not run along parallel with one another but they interlace and divide frequently and the branches

often join to form new nerve bundles which also usually divide and join again with other branches, and this may be repeated again and again. Where two nerves join, as in a plexus, there is not only a mixing of the nerve bundles of the two but a direct union of the funiculi so that when a funiculus from one has joined a funiculus from the other, the newly formed bundle surrounded by perineurium contains a mixed group of fibers from both. There may be a dozen or more bundles in a nerve bound together and surrounded by epineurium. The funiculi vary greatly in size, from minute threads to good-sized bundles. If all the epineurium could be dissected away, it would not be difficult to see how the funiculi branched and how the branches were joined together again. This can be done only with great difficulty if at all in the majority of embalmed bodies. In only a few instances, in selected cases, have I, by means of ordinary dissection been able to remove most of the epineurium and to trace the funiculi to any extent through a plexus. I have found that even with the greatest care in making such a dissection there were many places in which it was impossible not to break some of the fine connecting fascicles. Some of these connecting bundles are so small that it is difficult or absolutely impossible to distinguish them from the connective tissue. I have always felt much doubt as to whether I might not have broken without knowing it many of the minute bundles that pass from one funiculus to another. I have, therefore, not included the results of any such dissection in my series. Paterson ('96) also feels doubt of his ability to make such dissections, and he says "by anatomical methods it is impossible to separate the fibers of one spinal nerve from its neighbor."

In all of the cases included in this report the dissection has been carried only so far as seemed safe and only so much of the connective tissue has been removed as was possible without danger of tearing the nerve bundles. It was thought best not to attempt to remove all of the peripheral epineurium in any of the cases.

To make out the distribution of the fibers of a given spinal nerve in the branches of the brachial plexus it would be necesary

to follow these through the funiculi. To do this would necessitate the removal not only of the epineurium but also of the perineurium. I do not believe that this is anatomically possible. Some investigators have apparently been more successful in removing the epineurium and tracing nerve bundles and nerve fibers through the plexus. Their reported results indicate that they were able to follow not only the funiculi but the fibers of a given spinal nerve and that they could make out the distribution of the fibers to the various branches of the plexus.

SEPARATION OF NERVE BUNDLES BY MACERATION

In order to see how far it was possible to remove the connective tissue by chemicals and to trace back the nerves to their elemental constituents, I have experimented with various macerating fluids. That which I found the most satisfactory consisted of 20 parts of strong nitric acid and 20 parts glycerine in 40 parts water. A number of plexuses have been macerated in this fluid. A plexus placed in this macerating fluid was allowed to remain until the connective tissue of the epineurium was soft and pulpy and could be easily removed with a soft camel's hair brush. The nerves were allowed to remain in the fluid 48 hours or more depending on the method by which they had been embalmed and hardened. When the epineurium was sufficiently softened there was a decided shortening in the length of the plexus. This occurred suddenly when the maceration had been carried to a certain stage and not gradually as the maceration progressed. The cause of this contraction I have not been able to explain. When sufficiently macerated so that the epineurium could be easily removed, plexuses may be kept for almost any length of time in strong alum solutions or in 10 per cent formalin. As it is the connective tissue that gives the strength to the nerves they are, after maceration, easily broken and must be handled very carefully. Even if the maceration is continued for a much longer time, it does not soften the perineurium. This remains as a smooth definite sheath around the nerve bundles until with prolonged maceration the whole plexus becomes so pulpified and so softened that it cannot be studied at all. I do not know whether

the difference in the effect of the nitric acid on the epineurium and the perineurium is due merely to greater density of the latter, or if there is a difference in the kind of connective tissue forming each of them. It is clearly evident that when two funiculi join to form a third, we cannot, by this method, determine whether the branches from this latter contain nerve fibers from one or from both of the original bundles.

Figure 9 shows the principal funiculi of a plexus from which the epineurium has been removed by maceration. From this it will be seen how complicated is the network of bundles and how absolutely hopeless it is to attempt to trace to a definite spinal nerve the elements which enter into some of the branches even when the epineurium has been so completely removed. It will be seen, therefore, how much more difficult, in fact, how impossible it is to trace these funiculi; let alone nerve fibers accurately by means of ordinary dissection.

COMBINATION OF NERVES TO FORM A PLEXUS

We have so far been speaking of the brachial plexus, while as a matter of fact we have been dealing only with the nerves which go to make up that plexus. These nerves are usually described as combining to form the plexus in the following manner. The fifth and sixth cervical nerves unite to form a common stem, the cephalic trunk (upper or outer trunk or primary cord) and in the same way, the eighth cervical and first thoracic nerves unite to form a caudal trunk (lower or inner, trunk or primary cord) while the seventh cervical remains single and represents an intermediate trunk (middle, trunk or primary cord). Each of these trunks divides into ventral and dorsal branches. The ventral branches of the cephalic and intermediate trunks join to form a lateral fasciculus (outer cord); the ventral branch of the caudal trunk remains single as the medial fasciculus (inner cord); while the dorsal branches of all three trunks join to form the dorsal fasciculus (posterior cord) (figs. 1, 2, 3).

VARIATIONS IN THE FORMATION AND DIVISION OF THE TRUNKS AND OF THE FASCICULI OF THE PLEXUSES

Variations of the above arrangement fall into two main groups. In the first, no true cephalic or caudal trunks are formed but some or all of the nerves divide into dorsal and ventral branches and these combine to form the lateral, medial and dorsal fasciculi, or no true dorsal or lateral fasciculi are formed but branches from the dorsal and ventral rami of the nerves or trunks unite to form the branches of the plexus, or the cephalic and intermediate trunks fail to divide into dorsal and ventral branches but the trunks unite to form a single lateral cord which then divides into dorsal and ventral branches. In all of these variations the fasciculi or their branches receive fibers from the same spinal nerves as they would in the usual arrangement.

There is another group of variations of the plexus in which the lateral fasciculus receives fibers from nerves caudal to the seventh cervical or in which the medial fasciculus receives fibers from nerves cephalic to the eighth cervical nerve. A new element is, in these cases, introduced into either the lateral or medial fasciculus. These therefore are distinctly different than the usual and warrant the subdivision of the groups into subgroups or types. There are only 11 such atypical plexuses or 6.28 per cent of the 175 studied.

We shall consider the first group of variations, dealing with each trunk and fasciculus separately, and shall then consider the subdivision of the plexuses into subgroups.

The cephalic trunk

The cephalic trunk is formed by the union of the fifth and sixth cervical nerves in 157 plexuses or in 89.71 per cent of the 175 plexuses studied. The fourth cervical nerve in all cases where this enters the plexus joins the fifth before this has united with the sixth. In 153 of the 157 cases the cephalic trunk divides into dorsal and ventral divisions, (fig. 1), but in 4 it does not divide but is joined by the intermediate trunk and the nerve cord thus formed then divides into dorsal and ventral divisions (fig. 21).

This variation is perhaps caused by the dissector removing less than the usual amount of the connective tissue sheath that surrounds the trunks but at the time of the dissection it appeared as if as much had been removed as in other cases.

In 14 plexuses the fifth and sixth cervical nerves divide into dorsal and ventral branches and the ventral branches join to form a cephalo-ventral trunk (fig. 15). In 2 cases the fifth nerve divides into dorsal and ventral branches and the ventral branch joins the sixth nerve to form the cephalic trunk, and in 2 others the sixth nerve divides into dorsal and ventral branches and the ventral branch joins the fifth nerve to form the cephalic trunk. The cephalic trunk in these cases divides into dorsal and ventral divisions in the usual way. These cases are probably caused by the dissector removing more than the usual amount of the connective tissue sheath from around the trunks.

The intermediate trunk

The intermediate trunk is formed by the seventh cervical nerve only in all of the 175 cases. In 164 or 93.71 per cent it divides into dorsal and ventral branches (fig. 1). The ventral branch joins the ventral branches from the cephalic trunk or nerves to form the lateral fasciculus and the dorsal division joining the dorsal divisions of the cephalic and caudal trunks or the nerves forming them to form the dorsal fasciculus or its equivalent.

In 5 instances the intermediate trunk divides into 3 parts. In 4 of these, two of the divisions are ventral, one joining the ventral branch of the cephalic trunk to form the lateral fasciculus and the other passing to the medial fasciculus, while the dorsal branch goes into the dorsal fasciculus in the usual way (fig. 4). In the fifth instance there are two dorsal branches both of which go to the dorsal fasciculus, and the ventral branch joins the ventral branch of the cephalic trunk to form the lateral fasciculus. In 4 other plexuses, already noted in discussing the cephalic trunk, the intermediate and cephalic trunks join before dividing into dorsal and ventral branches (fig. 21). In 2 other cases the dorsal branch of the cephalic trunk joins the

intermediate trunk before this divides into dorsal and ventral branches (fig. 24).

The caudal trunk

The caudal trunk is formed by the union of the first thoracic and the eighth cervical nerves in 166 of the 175 plexuses or in 95.42 per cent (fig. 1). It then divides into dorsal and ventral divisions in 165 of them. In the other case the caudal trunk divides into the ulnar and the medial head of the median nerve and this gives off a dorsal branch. In 4 of the above 166 cases the eighth cervical nerve before joining the first thoracic gives off a small branch. This joins the lateral fasciculus of the plexus in 2 (fig. 27), the lateral head of the median nerve in 1 (fig. 25) and the ventral division of the intermediate trunk in 1. In 2 of the 166 the caudal trunk, before dividing into dorsal and ventral branches, gives off a small ventral ramus that joins the intermediate trunk in 1 (fig. 6), and the lateral fasciculus of the plexus in the other (fig. 8).

In 6 plexuses, the eighth cervical nerve divides into dorsal and ventral branches. The ventral branch joins the first thoracic nerve to form the medial fasciculus. There is no dorsal branch of the first thoracic (fig. 26). There is no caudal trunk that divides into dorsal and ventral divisions.

In 2 instances, both the eighth cervical and first thoracic nerves divide into dorsal and ventral branches and the ventral branches join to form the medial fasciculus. These and the proceeding 6 cases are perhaps produced by the dissector removing more than the usual amount of the connective tissue sheath, and it is not impossible that nerve fibers may have been broken through although no broken ends were found at the time the plexus was verified.

In one plexus the eighth cervical and first thoracic nerves join to form the caudal trunk which after receiving a branch from the intermediate trunk divides into dorsal and ventral divisions.

The lateral fasciculus of the plexus

The lateral fasciculus of the plexus is formed by the junction of the ventral divisions of the cephalic and intermediate trunks

in 143 plexuses, or in 81.71 per cent of the 175 studies (fig. 1). In two others, the intermediate trunk, before giving off its ventral division, receives a small additional branch from the caudal trunk. In 3 additional plexuses, the lateral fasciculus is formed in the usual way but receives a small additional branch, in 1 from the caudal trunk (fig. 8) and in 2 from the eighth cervical nerve (fig. 27).

In 2 other plexuses, the arrangement differs from the usual in that the ventral division of the intermediate trunk to the lateral fasciculus is given off after the trunk is joined by the dorsal division from the cephalic trunk (fig. 24). In 4 others the cephalic and intermediate trunks join and this cord then splits into a lateral fasciculus and a branch to the dorsal fasciculus (fig. 21). In the above 6 cases perhaps if more of the connective tissue had been removed from around the nerve they would have corresponded to the usual type.

In 12 plexuses, the fifth and sixth cervical nerves divide into dorsal and ventral branches and the ventral branches unite to form a cephaloventral trunk which is then joined by the ventral branch of the seventh cervical nerve to form the lateral fasciculus. In 9 of these cases, the fourth cervical nerve joins the fifth before it divides into dorsal and ventral branches.

There are 2 other cases, similar to the above except that the ventral branch of the sixth joins the undivided fifth cervical and the ventral branch of the cephalic trunk thus formed joins the ventral branch of the seventh cervical to form the lateral fasciculus.

In 7 cases there is no lateral fasciculus formed. In 4 of these the ventral branch of the cephalic trunk, and in 2 others a trunk formed by the union of the ventral branches of the fifth and sixth cervical divides into musculo-cutaneous and the lateral head of the median nerve (fig. 15). In 6 of the above this latter is joined by the ventral branch of the seventh cervical (fig. 16). In the other case, the ventral branch of the seventh cervical joins the medial head of the median, it corresponds to the lateral head of the ulnar (fig. 15). In the 21 plexuses just described, it is probable that more of the connective tissue was removed than usual and in the last 7 it is not impossible that small connecting

funiculi may have been broken, but no evidence of this was found by naked eye examination.

The medial fasciculus of the plexus

The medial fasciculus of the plexus is formed of the ventral branch of the caudal trunk in 166 or in 94.85 per cent of the 175 plexuses (fig. 1). In 5 of these there is a branch from the seventh cervical nerve to the medial fasciculus (fig. 5).

In 6 specimens the medial fasciculus is formed of the ventral branch of the eighth cervical joined with the whole of the plexus part of the first thoracic which has in these no dorsal branch; it takes the place of the caudal trunk (fig. 26). In one of these the medial fasciculus receives a branch from the seventh cervical nerve (fig. 4).

In 2 others the medial fasciculus is formed by the union of the ventral branches of the eighth cervical and first thoracic nerves. It is not impossible that there would be others arranged like the above if more of the connective tissue could have been safely removed.

There is one other specimen in which the medial fasciculus and the caudal trunk are identical. The medial head of the median in this gives off a dorsal branch to the dorsal fasciculus which is usually given off from the caudal trunk.

The posterior fasciculus of the plexus

The posterior or dorsal fasciculus of the plexus is formed of the dorsal branches of the cephalic, intermediate and caudal trunks, or of dorsal branches from the nerves making up these trunks. It is seldom possible to trace the branches proximally to the spinal nerves. These dorsal branches do not always combine in the same way.

In 10 plexuses (fig. 25) the dorsal branches of all these trunks meet at about the same point to form the posterior fasciculus (fig. 1).

In 86 plexuses the dorsal branches of the cephalic and intermediate trunks join to form a cephalodorsal cord which is joined

somewhat more distally by the dorsal branch of the caudal trunk (fig. 2). In 2 of these the dorsal fasciculus receives an additional branch or branches. In 1 this comes from the ventral division of the intermediate trunk and is single, in the other it comes from the medial fasciculus and is double. In 5 others, of the above plexuses, one of the branches to the dorsal fasciculus receives an additional branch. This joins the dorsal branch from the cephalic trunk in 3 and comes from the sixth cervical nerve in 2 and from the fifth in 1. It joins the dorsal branch from the intermediate trunk in 1 and arises from the eighth cervical nerve and it joins the dorsal branch from the caudal trunk in 1 and comes from the intermediate trunk. In 2 of the above 86 plexuses the branch from the caudal trunk receives no fiber from the thoracic nerves, but comes from the eighth cervical only.

In two cases the dorsal division of the cephalic trunk joins the intermediate trunk and the cord thus formed divides into dorsal and ventral parts. The dorsal division joins with the dorsal division of the caudal trunk to form the dorsal fasciculus (fig. 24).

In 9 cases the dorsal branches of the fifth and sixth cervical nerves combine to form a dorsocephalic trunk, the equivalent of the dorsal division of the cephalic trunk; this then joins the dorsal division of the intermediate trunk and more distally is joined by the dorsal division of the caudal trunk.

There is another case exactly like this except that the caudal trunk does not divide into dorsal and ventral branches but after giving off the cutaneous branches splits up into ulnar and medial head of the median nerve and this latter gives off a dorsal branch that takes the place of and is equivalent to the usual dorsal branch of the caudal trunk.

In 26 plexuses the dorsal divisions of the intermediate and caudal trunks join to form a caudodorsal cord which is joined more distally by the dorsal division of the cephalic trunk (fig. 5). In three of these the posterior fasciculus receives an additional branch, in one from the lateral fasciculus, in two from the medial. In one of the above cases the branch from the caudal trunk comes from the eighth cervical only. In one, the dorsal branch of the

intermediate trunk receives an additional branch from the ventral branch of the cephalic trunk.

In 4 instances the cephalic and intermediate trunks join and the cord thus formed divides into dorsal and ventral branches. The dorsal branch joins with the dorsal division of the caudal trunk to form the posterior fasciculus (fig. 21).

In one case the fifth and sixth cervical nerves divide into dorsal and ventral branches. The dorsal branches unite to form a dorsocephalic cord that joins another cord formed by the union of the dorsal branches of the intermediate and caudal trunks to form the posterior fasciculus. Slightly more distal the posterior fasciculus receives an extra branch from the lateral fasciculus. It will be seen then that the posterior fasciculus is formed by the union of dorsal branches of the plexus in 139 records.

In 36 plexuses or 20.57 per cent of the 175 studied, there is no real posterior fasciculus unless we consider a single nerve, the radial, as representing the dorsal fasciculus.

The arrangement of the dorsal branches in these cases will be described in connection with the discussion of the radial and axillary nerves.

If the epineurium could have been completely removed from all the plexuses, I have no doubt that more of them would have corresponded to this last group in which no real dorsal fasciculus was formed. It will be noted that in all but 6 of the plexuses of this series the caudal branch to the dorsal fasciculus was from the caudal trunk formed by the eighth cervical and the first thoracic nerves. Herringham ('87) found the branch from the first thoracic to the dorsal fasciculus absent in 39 out of 45 cases or in 86.66 per cent. W. Harris ('04) however, found the branch from the first thoracic nerve to the dorsal fasciculus 7 times out of 9. In the specimens studied by maceration in nitric acid, I found that the first thoracic sends branches to the dorsal fasciculus in the majority of instances. As already explained, because of the danger of breaking the minute connections by gross dissection I have attempted to determine in only a few instances whether or not the branch to the dorsal fasciculus from the caudal trunk contained fibers from both the eighth cervical and first

thoracic. I do not believe that with my material this point could have been determined in the majority of plexuses.

SUBDIVISIONS OF THE PLEXUSES OF GROUPS 1, 2 AND 3

The lateral fasciculus of the plexus or its equivalent contains fibers from the seventh cervical and the nerves cephalic to this and the medial fasciculus contains fibers from the eighth cervical and the nerves caudal to this in 164 or 93.71 per cent of the 175 plexuses studied. Some of the 11 atypical specimens are found in each of the three groups into which the plexuses are divided. In 6 of the 11 atypical specimens there is a branch from the intermediate trunk (seventh cervical nerve) to join the caudal trunk or the medial fasciculus of the plexus (fig. 4). In the other 5 there is a branch from the caudal trunk or the eighth cervical nerve to join the lateral fasciculus or one of the nerves that go to it (figs. 6 and 8).

The branch to the lateral fasciculus is found in the plexuses of group 1 and 3 but has not been found among those of group 2. The branch from the intermediate trunk to the medial fasciculus has been found among groups 1 and 2.

Schumacher describes in 8 of the 10 cases examined by him a branch from the lateral to the medial fasciculus. He may refer to a branch similar to the one mentioned above from the intermediate trunk to the medial fasciculus or to a branch similar to the lateral head of the ulnar nerve.

It is not improbable that the significance of this branch to the medial fasciculus may be the same as that of the lateral head of the ulnar nerve, although in one case the two coexist. Its sole function might be to bring fibers of the seventh cervical or some nerve cephalic to this to the ulnar nerve.

The lateral head of the ulnar nerve occurs in 75 plexuses or in 42.85 per cent of the 175. Fifty-five of these are in group 1 or 50.00 per cent of the 110 plexuses in the group. Sixteen are in group 2 or 30.76 per cent of the 52 plexuses of this group and 4 are in group 3 or in 30.76 per cent of the 13 plexuses found here. It will be seen then that this also occurs more often in the cephalic group of plexuses. A more detailed description of this lateral

ulnar fasciculus will be given in connection with the ulnar nerve.

Those plexuses within each group in which branches from the seventh cervical (the intermediate trunk) contribute to the medial fasciculus I have classified as cephalic types and those in which a branch from the caudal trunk goes to the lateral fasciculus either directly or by joining the seventh cervical nerve I have classified as caudal types.

Group 1 is in this way subdivided into three subgroups; group 2 into two subgroups, and group 3 into two subgroups. The types in group 1, I have classified A, B, and C. Type B (fig. 1) is the typical arrangement described above. Type A is the most cephalic and type C the most caudal. In all of these in group 1 the fourth cervical nerve sends a branch to the plexus.

Type A

Type A is distinguished by having a ventral branch from the intermediate trunk (seventh cervical nerve) to the medial fasciculus of the plexus so that this contains fibers from the seventh and eighth cervical and first thoracic nerves (fig. 5). The lateral fasciculus contains fibers from the fourth, fifth, sixth and seventh cervical nerves. The plexuses of this type are the most cephalic of the whole series. There are five cases of this type or 2.85 per cent of the 175 plexuses. In one of the cases the branch from the caudal trunk to the dorsal fasciculus is from the eighth cervical nerve alone, so that the dorsal fasciculus has no branch from the first thoracic.

Type B

In type B the lateral fasciculus contains fibers from the fourth, fifth, sixth and seventh cervical nerves and the medial fasciculus fibers from the eighth cervical and first thoracic. There are 101 cases of this type, or 57.71 per cent of the 175 plexuses, that is, more than half the total number of plexuses are of type B (fig. 1).

Type C

Type C differs from the preceding in that there is a branch from the caudal trunk to join the seventh cervical nerve or the

lateral fasciculus (fig. 6). The lateral fasciculus then receives fibers from nerves caudal to the seventh. The medial fasciculus is formed from the eighth cervical and first thoracic nerve. There are 4 cases of this kind, or 2.28 per cent of the 175 plexuses. In 3 of the 4 cases, the extra branch to the lateral fasciculus comes from the eighth nerve only (fig. 27). In the exceptional instances it arises from the caudal trunk. In this case the branch joins the seventh cervical before it divides into dorsal and ventral branches. In another case the branch joins the ventral branch of the seventh cervical. In the other two cases, the branch goes to the lateral fasciculus directly. In two cases the branch also sends an additional offshoot to the dorsal fasciculus. In both cases where the branch to the lateral fasciculus sends a branch to the dorsal fasciculus there is also another separate branch from the caudal trunk to the dorsal fasciculus.

The subgroups of group 2 I have designated types D and E. They receive no fibers from the fourth cervical nerve.

Type D

In type D there is a branch from the seventh cervical nerve to the medial fasciculus of the plexus (fig. 7). The medial fasciculus is then formed of fibers from the seventh and eighth cervical and first thoracic nerves and the lateral fasciculus from the fifth, sixth and seventh cervical. This type is exactly like type A except for the absence of the branch from the fourth cervical nerve. There is only one case of this type or 0.57 per cent.

Type E

In type E the lateral fasciculus is formed of fibers from the fifth, sixth and seventh cervical nerves and the medial fasciculus of branches from the eighth cervical and first thoracic nerves (Fig. 2). The plexuses of this type are exactly like the plexuses of type B except that the branch from the fourth cervical nerve does not join them. There are 51 plexuses of this type or 29.14 per cent of the 175 plexuses.

Group 3 plexuses were divided into types F and G. The whole of the fifth cervical does not contribute to this type.

Type F

In type F the lateral fasciculus is formed of branches from the fifth, sixth and seventh cervical nerves and the medial of branches from the eighth cervical and first thoracic nerves (fig. 3). The plexuses of this type are exactly like those of types B and E except that the whole of the fifth cervical nerve does not enter the plexus. There are 12 plexuses of this type or 6.85 per cent of the 175. In one of the plexuses that I have classified as belonging to this type there is a small branch from the fourth cervical nerve to the fifth, in addition to the branch from the fifth to the fourth (fig. 21). This latter branch is larger than the branch from the fourth to the fifth, and I have therefore considered the plexus as belonging to group 3, type F. Types B, E, and F correspond to the type and differ from one another exactly as groups 1, 2 and 3 differ, that is, B receives a branch from the fourth and E does not, and in F the fifth cervical sends a branch to the fourth.

Type G

In type G there is a branch from the caudal trunk to the lateral fasciculus (fig. 8), just as there is in type C. Types C and G differ from one another in that in C there is a branch from the fourth cervical entering the plexus and in G there is no such branch but there is a branch from the fifth to the fourth cervical nerve. There is only 1 case of this kind making 0.57 per cent of the 175 plexuses studied.

SYMMETRY AND ASYMMETRY ON THE TWO SIDES OF THE BODY

As already pointed out a considerable number of the plexuses of my series were from only one side of the body. There are, however, 63 bodies in which there are satisfactory records for both sides. In 39 of these, or 61.90 per cent of them, the type of plexus is the same on both right and left sides, while in the remaining 24 the type of plexus is asymmetrical.

In these I have tried to determine if the asymmetry is more frequently found in white or colored, in male or female bodies.

Where asymmetry occurred I have tried to determine if this is more common among one type of plexus than another, or if sex or color influences it; also if one type of plexus is found more often on one side of the body than the other.

Symmetry in males and females, white and colored

There are 25 male bodies in which the plexuses on both sides are of the same type, or 62.50 per cent of the 40 male bodies.

There are 15 male bodies in which the type of plexus is asymmetrical, or 37.50 per cent.

There are 14 female bodies in which the plexuses on both sides are of the same type, or 60.86 per cent of the 23 female bodies. In 9 female bodies the plexuses on the two sides are of different types or 39.13 per cent.

There are 18 white bodies with symmetrical plexuses and 14 in which they are asymmetrical, or 56.25 per cent of the 32 white bodies have symmetrical plexuses and 43.75 per cent have the plexuses asymmetrical.

There are 21 colored bodies in which the plexus is of the same type on the right and left sides, or 67.74 per cent of the 31 colored bodies. In 10 colored subjects or 32.25 per cent, the plexuses are asymmetrical.

From the above it will be seen that symmetry is more common than asymmetry in about the ratio of 3 to 2. It would appear also to be very slightly, 1.64 per cent, more common in males than in females.

Symmetrical arrangements of plexuses was more common in the colored than in the whites by about 11.5 per cent.

There are 13 white males with asymmetrical arrangement of the plexuses, or 54.16 per cent of the 24 white males, and 11 or 45.83 per cent in which the type of plexus is different on the two sides of the body. There are 12 colored males with symmetrical and 4 with asymmetrical plexuses or 75 per cent and 25 per cent respectively of the 16 colored males. Symmetry is found in 3 cases to every one in which there is asymmetry among the colored males.

There are 5 white female bodies with symmetrical arrangement and 3 with asymmetrical, or 62.50 per cent symmetrical and 37.50 asymmetrical among the 8 white females. In the 9 colored female bodies, there is a symmetrical arrangement and in 6, or 60 per cent and in 40 per cent, an asymmetrical. This gives a ratio of 3 to 2 among the colored females.

A symmetrical arrangement was found most often among colored males, least often among white males, and more often among white than among colored females. The ratio of symmetry to asymmetry was in colored males 3 to 1, in colored females, 3 to 2. Symmetry was 8.33 per cent more common than asymmetry in white males and 25 per cent more common in white females.

Symmetry as regards the type of plexus

Among the 63 bodies where I have satisfactory records from both sides of the body there are only 4 in which the type A plexuses are found. All are asymmetrical. In two of them type A is on the right side, 1 white male and 1 white female. In two, type A is on the left, both in colored females. It is interesting to note that in both the cases where the B type of plexus is on the right side there is a lateral head to the ulnar nerve on this side. In the cases where the B type is on the left side, there is no such branch.

There are 76 plexuses of the B type and in 54 of these it occurred on both sides of the body, or in 71.05 per cent. In the 22 bodies in which the B type is found only on one side, the A type is found on the other side in 4, 2 right and 2 left, type C is on the left in 2, the E type is on the left in 7 and on the right in 6, and the F type on the left in 3. It will be noted then that in 14 of the above the B type is on the right and in 8 on the left or nearly 2 to 1.

There are 2 records of C type plexus both on the left side of white males and the type B plexus is on the right.

There is but one record of type D. This is on the left of a white male and the type E plexus is on the right.

There are 33 plexuses of the E type, 18 of these are on both sides of the body, or in 54.54 per cent. The remaining 15 plexuses of the E type are 8 on the right and 7 on the left. Those on the right are associated in 6 cases with B, in 1 with D, and in 1 with F type of plexus on the left. In the 7 instances in which the E type is on the left, the B type is on the right.

Of the 10 plexuses of the F type 6 are found on both sides or 60 per cent. The other 4 cases are associated with B type on the right in 3 and E type on the right in 1.

Symmetry occurs only in the B, E and F types of plexus, which are the most common and typical plexuses for each of the three groups, 1, 2 and 3. This is suggestive that the A, C and D types of plexus are anomalous and may have no great significance. There is no plexus of the G type in the bodies where the records for both sides are complete. It will be noted that when the A, C or D type of plexus occurs on one side it is associated with a plexus in the same group on the other side, A and B, B and C, E and D.

Slightly over 71 per cent of the B type plexuses are symmetrical, 60 per cent of the F type and a little over 54 per cent of the E type. That is, in the most cephalic group of plexuses symmetry occurs most often and in the intermediate group least often.

Among the 24 bodies with an asymmetrical arrangement of the plexus, a more cephalic type is found upon the right side in 15 or 62.50 per cent and a more caudal type in 9 or 37.50 per cent.

Among the 24 bodies with asymmetrical plexuses, group 1 is found on one side and group 2 on the other in 13 instances or 54.16 per cent. In these type B of group 1 is on the right side in 7 and type E of group 2 on the left. In the other 6 cases, the arrangement is reversed. In 3 others or 12.50 per cent group 1 is on the right side and group 3 on the left. In these type B of group 1 is on the right and type F of group 3 on the left. In another instance group 2 is on one side and group 3 on the other. In this case type E of group 2 is on the right and type F of group 3 on the left. The remaining 7 bodies were classed as asym-

metrical because different types of plexus are found on the two sides but both types are in the same group. Six are in group 1 and 1 in group 2. Of those in group 1, in 4 type A is on one side and type B on the other. In 2 of these type A is on the right and in 2 it is on the left. In two bodies type B is found on the right, type C upon the left. In the case where both plexuses are in group 2, type E is on the right and type F on the left.

THE ORIGIN OF THE BRANCHES FROM THE BRACHIAL PLEXUS

As will be seen from figures 1 to 8 some of the branches of the plexus arise from the rami of the spinal nerves forming the plexus, some from the cephalic and caudal trunks and some from the medial, lateral, and posterior fasciculi. In my study of the nerves of the plexus, the trunks and fasciculi were not broken up and subdivided so that one could see just which spinal nerve contributed to each branch, and as demonstrated by my maceration experiments, this is impossible in a great many cases even by this method, let alone by dissection. It is assumed then that if a nerve arises from a trunk formed by branches from two or more spinal nerves that both or all of them may send fibers to the nerve. It is recognized, of course, that in some cases only one of the nerves may contribute to a branch, but there is no way of determining by anatomical methods when this occurs or which nerve it is. It seems best, therefore, to consider all of the nerves that help to form a trunk or fasciculus as potential elements in each branch from the trunk or fasciculus.

In the usual descriptions, the lateral fasciculus is said to terminate by dividing into the musculocutaneous nerve and the lateral head of the median nerve; the medial fasciculus by dividing into the ulnar nerve and the medial head of the median nerve; the posterior fasciculus by dividing into the axillary and radial nerves. It has already been shown that this does not always occur but I shall not deal separately with the percentage occurrence of this division of each of these fasciculi into medial and lateral branches but shall consider it in connection with each of the branches.

THE ULNAR NERVE

The ulnar nerve is usually described as arising from the medial fasciculus of the plexus, and the medial fasciculus of the plexus is usually stated to be formed by the junction of the eighth cervical and first thoracic nerves and also at times to receive fibers from the second thoracic nerve.

In my series, the ulnar nerve is formed by the division of the medial fasciculus of the plexus into ulnar nerve and medial head of the median nerve, in 170 of the 175 plexuses studied, or in 97.14 per cent (figs. 1–8).

In 5 instances, the ulnar nerve arises from a trunk formed by the union of the medial fasciculus of the plexus with the whole or a part of the lateral fasciculus. In 3 of these the trunk formed by fusion of the medial and lateral fasciculi of the plexus almost immediately breaks up into the ulnar, median, and musculocutaneous nerves (fig. 27). In the other 2 cases, the lateral head of the median joins the medial fasciculus of the plexus and the trunk thus formed divides into ulnar and median nerves (fig. 28).

The lateral head of the ulnar nerve

In some of the 170 plexuses in which the ulnar nerve arises by division of the lateral fasciculus of the plexus, the nerve receives on its lateral side an additional branch. This has been named the lateral head of the ulnar nerve, caput laterale nervus ulnaris (figs. 1, 2, 3, 10). This lateral head of the ulnar nerve has been figured and mentioned at intervals for a long time by various authors.

The size of the lateral head of the ulnar nerve is subject to much variation, ranging from a minute thread to a good sized branch, as large as the usual medial antibrachial cutaneous nerve. It is often small and pierces or crosses the medial head of the median nerve. There it is intimately bound up in the connective tissue sheath of the latter so that its relation to the ulnar nerve is easily overlooked. In separating the ulnar from the median nerve in dissection, the connection of the lateral head of the ulnar, if not particularly looked for, is often broken.

Walsh ('77) personally examined 350 plexuses and entirely overlooked this branch in the first 60 but once his attention was directed to it he found it absent in only 25 of the remaining 290.

The dissections in my series were made by students who were not especially looking for a lateral head of the ulnar nerve, and although the work was carefully done I feel quite confident that in a number of cases there had been a lateral head to the ulnar which could not be demonstrated at the time the plexus was examined by the investigator since the connection with the ulnar had been broken in the dissection and the broken ends could not be found.

In some of these cases there remains a branch connecting with the medial head of the median nerve and similar in all respects to a lateral head of the ulnar nerve except that the connecting fibers could not be demonstrated (fig. 34). There are 30 such cases in my series. Walsh, however, found but 10 instances in which a similar branch failed to send fibers to the ulnar nerve but ended exclusively in the medial head of the median nerve.

There are 8 other instances in which there is a branch which joins the medial fasciculus of the plexus close to the point where this divides into the ulnar and the medial head of the median nerve. I have not been able to prove in these 8 cases that fibers from this branch go to the ulnar nerve, but this is probably the lateral head of the ulnar nerve since in all other similar cases I have found by very careful dissection or by macerating the plexuses in nitric acid that this branch sends fibers to the ulnar nerve. Sometimes all of its fibers go to the ulnar nerve and sometimes part to the ulnar and part to the medial head of the median nerve. I therefore believe this branch always represents the lateral head of the ulnar nerve and have so considered it in this paper. In 6 of the above cases this branch arises from the lateral fasciculus of the plexus (fig. 11) and in 2 from the seventh cervical only.

The lateral head of the ulnar nerve, in my series, passes, in 40 cases, as a single branch to the ulnar nerve (fig. 2), but in 28 cases it divides and sends one or more branches to the medial

head of the median (fig. 3). In the 8 in which it passes to the medial fasciculus, the ulnar alone, or both ulnar and median nerves may be supplied by it. The fibers which pass to the medial head of the median are usually collected into a single fasciculus (figs. 1, 3, 8) but in a few cases they are divided into 2 or more small branches (fig. 14).

In its course to the ulnar nerve the lateral head of the ulnar in some cases passes dorsal to the medial head of the median (fig. 1), in a few it passes ventral to the medial head of the median, but in the most usual arrangement, it pierces the medial head of the median (figs. 2, 3 and 8).

In my series the lateral head of the ulnar arises from the lateral fasciculus of the plexus in 40 cases (figs. 1 and 3), in 3 of these it passes to the medial fasciculus of the plexus close to its division into the ulnar nerve and medial head of the median nerve (fig. 11), in 13 to the ulnar nerve only (fig. 13), and in 24 to the medial head of the median nerve as well as to the ulnar (figs. 1 and 3). In 2 of these instances the lateral head of the ulnar gives also a branch to the lateral head of the median (fig. 24).

The lateral head of the ulnar nerve arises from the seventh cervical nerve in 13 cases, in 2 of these passing to the medial fasciculus of the plexus, in 3 to the ulnar nerve only and in 8 to the medial head of the median nerve as well as to the ulnar nerve (fig. 10). In 4 of these the lateral head of the median nerve is formed in the usual way but in the other 4 it is irregular as described in connection with the median. In 2 of these the seventh cervical forms the lateral head of the median nerve (fig. 17), so the lateral head of the ulnar in these instances arises from the lateral head of the median.

In 22 cases the lateral head of the ulnar arises from the lateral head of the median nerve, in 21 of these it passes to the ulnar only (fig. 2), in one case to both the ulnar and the medial head of the median nerve (fig. 8).

In the series reported by Walsh the lateral head of the ulnar arose proximal to the middle of the lateral fasciculus of the plexus in 156 plexuses, in the rest more distally, in 30 arising from the lateral head of the median nerve.

The lateral head of the ulnar nerve is found in 75 of the 175 plexuses of my series or in 42.85 per cent of them. Fifty-five of these, or 31.42 per cent were group 1 plexuses, 16 or 9.4 per cent were group 2 plexuses and 4 or 3.4 per cent were group 3 plexuses. The lateral head of the ulnar nerve is found in 55 of the 110 plexuses of group 1 or in 50 per cent of them, in 16 of the 52 plexuses of group 2 and in 4 of the 13 plexuses of group 3 or 30.76 per cent of the group in each of these. If, however, we entirely disregard the 30 doubtful cases in which a similar branch has not been proved to join the ulnar but ends in the medial head of the median nerve (fig. 20), we have a lateral head of the ulnar occurring in 75 out of 145 cases or in 51.72 per cent of the cases examined. If, on the other hand, we consider this branch to the medial head of the median as representing the lateral head of the ulnar, which I believe in the majority of cases it does, we have a lateral head of the ulnar occurring in 105 of the 175 plexuses or 60.00 per cent, which I believe to be more nearly the true percentage.

Walsh ('77), in an examination of 290 plexuses, found the lateral head of the ulnar nerve absent in but 25 cases and in 13 of these there was in place of it a branch arising from the seventh cervical nerve above the clavicle which joined the medial fasciculus of the plexus high up in the axilla.

Spinal nerves that send fibers to the ulnar nerve

The ulnar nerve probably, in all cases, receives fibers from the eighth cervical and first thoracic and in those cases where the second thoracic enters the plexus the possibility of this nerve sending fibers to the ulnar nerve cannot be excluded.

Besides the eighth cervical and first thoracic (or first and second), fibers from the seventh cervical nerve may enter the ulnar nerve in plexuses of types A and D. For, as has been shown, fibers from the seventh cervical nerve pass to the medial fasciculus of the plexus through the branch which is the distinguishing characteristic of these types, and since the ulnar nerve arises from the medial fasciculus, we cannot be sure that the seventh cervical nerve does not take part in its formation.

Through the lateral head of the ulnar there is brought to the ulnar nerve fibers from the seventh cervical nerve and, in cases where the branch arises from the lateral fasciculus of the plexus or from the lateral head of the median nerve, the possibility of fibers from the sixth, fifth and fourth cervical nerves, one or all, entering the ulnar nerve cannot be excluded. Since this branch brings to the ulnar nerve fibers from the seventh cervical nerve, we might expect to find it absent in those cases in which these fibers may be obtained in another way, for example, in those cases in which the seventh cervical nerve sends a branch to the medial fasciculus of the plexus as in plexuses of types A and D. In 5 of the plexuses of these two types there is no lateral head to the ulnar nerve. In one plexus of type A, there is however also a lateral head to the ulnar nerve (fig. 12), but in this case it arises from the lateral fasciculus of the plexus and may contain fibers from some of the nerves cephalic to the seventh cervical. It is suggestive that the branch from the seventh cervical nerve to the medial fasciculus in the 6 plexuses of types A and D represents the lateral head of the ulnar arising more proximally than usual although in three of the 6 instances the fibers of the seventh cervical nerve cannot be excluded from one of the other branches of the plexus.

In the 5 atypical cases in which the medial fasciculus joins the lateral or the lateral head of the median before the ulnar nerve is given off, the ulnar may receive fibers from any of the nerves cephalic to the eighth cervical. If we disregard the 30 cases in which a branch similar to the lateral head of the ulnar is present but ends in the medial head of the median nerve and that I have considered as a possible broken branch, we have 145 plexuses in which the record is positive. Among these the ulnar nerve is formed of fibers of the eighth cervical and first thoracic nerves in 61 or 42.06 per cent and of fibers from the seventh and eighth cervical and first thoracic in 16 or 11.03 per cent. In the remaining 68 cases or 46.89 per cent the ulnar has a lateral head that comes from the lateral fasciculus of the plexus, or from the lateral head of the median, and it is not possible to say if the fibers are from the seventh cervical alone

and that there may not be fibers from the nerves cephalic to the seventh cervical. The 5 cases in which the lateral fasciculus does not give off the ulnar until after it has joined the medial fasciculus or the medial head of the median are included here also.

There are 92 satisfactory records of the ulnar nerve among the group 1 type of plexus. In 52 of these or 57.28 per cent of the group the fourth, fifth, sixth, seventh and eighth cervical and the first thoracic nerves may take part in the formation of the ulnar nerve. In eleven cases, 11.95 per cent of the group, the seventh and eighth cervical and first thoracic nerves may send fibers to the ulnar nerve and in 29 plexuses, 31.52 per cent of the group, the eighth cervical and first thoracic only send fibers to the ulnar nerve.

There are 47 satisfactory records of the ulnar nerve among the plexuses of group 2. In 14 of these, 29.78 per cent of the group, the fifth, sixth, seventh and eighth cervical and first thoracic nerves may take part in the formation of the ulnar nerve. In 4 cases, 8.51 per cent of the cases, the seventh and eighth cervical and first thoracic alone may send fibers to the ulnar nerve and in 29 plexuses, 61.70 per cent of the group, the eighth cervical and first thoracic nerves alone may send fibers to the ulnar nerve.

There are 6 satisfactory records of the ulnar nerve among the group 3 plexuses. In 2 of these, 33.33 per cent of the group, the fifth, sixth and seventh cervical nerves in addition to the eighth cervical and first thoracic nerves may take part in the formation of the ulnar nerve. In 1 case, 16.66 per cent of the group, the seventh and eighth cervical and first thoracic nerves send fibers to the ulnar nerve and in 3 plexuses, 50 per cent of the group, the eighth cervical and first thoracic nerves alone make up the ulnar nerve.

It will be seen from the above that in the most cephalic group of plexuses there is a relatively greater percentage of the group in which in addition to the eighth cervical and first thoracic nerves, the fourth, fifth, sixth and seventh cervical nerves may also take part in the formation of the ulnar nerve and in the more caudal type, that is, groups 2 and 3, a relatively greater

percentage of each group in which only the eighth cervical and first thoracic nerves enter and the more cephalic nerves take no part in the formation of the ulnar nerve.

Wichmann ('00) has tabulated from the notes of Renz the reports of 24 writers on 171 cases, showing the spinal nerves that contribute to the formation of the ulnar nerve.[4] Since then, Schumacher ('08) has reported on 10 more investigated by him. If my 145 plexuses are added to these we have a total of 326 cases.

The eighth cervical and first thoracic contribute to the ulnar nerve in 103 cases or 31.28 per cent, Wichmann 41, Schumacher 1, Kerr 61.

The seventh and eighth cervical and first thoracic nerves send fibers to the ulnar nerve in 114 cases or 35.27 per cent, Wichmann 89, Schumacher 9, Kerr 16.

In addition to the eighth cervical and first thoracic nerves the ulnar may receive a contribution from the fifth, sixth and seventh cervical nerves in 87 cases or in 26.68 per cent, Wichmann 19, Kerr 68. The fourth cervical nerve also cannot be excluded in 52 of these from my series.

In Wichmann's tabulation there are 22 additional cases in which the ulnar nerve is formed in some other way. It arises from the eighth cervical nerve in 3 instances, from the seventh and eighth in 3, from the sixth, seventh and eighth in 10 and from the fifth, sixth, seventh and eighth in 6.

From the above it will be seen that the ulnar nerve receives fibers from one or more of the nerves cephalic to the eighth cervical in 220 plexuses or in 67.48 per cent of the 326 cases. In the above tabulation I have excluded the 31 cases in which there is a branch to the medial head of the median. In some of these I feel sure the ulnar also receives fibers from this branch.

THE MEDIAN NERVE

The median nerve is usually described as formed by the union of two branches, one from the medial and one from the lateral fasciculus of the brachial plexus, the medial and lateral

[4] In Wichmann's tabulation he has credited Walsh 101 cases, 27 of these are apparently erroneously credited and should have been credited to some one else.

heads of the median nerve respectively. It will thus be seen that the nerve may contain fibers from all the spinal nerves entering the plexus.

In 150 of the 175 plexuses of my series, the median nerve is formed in the usual way by two heads, as described above (fig. 5). The lateral head arises from the lateral fasciculus of the plexus which is formed by the union of the ventral branch of the cephalic trunk with the ventral branch of the intermediate trunk (figs. 1, 2, 3). There are two other plexuses differing from the above only in that the lateral fasciculus of the plexus from which the lateral head arises receives fibers from the caudal trunk of the plexus. One of these plexuses belongs to type C (fig. 6), and the other to type G of plexus (fig. 8). There are then 152, or 86.85 per cent, of the 175 plexuses of my series in which the median nerve may be considered to arise by two heads in the usually described manner.

In the remaining 23 plexuses of the series, the median nerve is formed in a different way.

In 7 of these instances, the lateral head of the median is formed by the ventral branch of the cephalic trunk, after giving off the musculocutaneous nerve, joining with the ventral branch of the intermediate trunk (seventh cervical nerve) (fig. 18). In another case, the ventral branch from the intermediate trunk joins the medial head of the median, giving off also the lateral head of the ulnar (fig. 15), but sending no branch to the medial head of the median. In three of the above cases, the branch from the seventh cervical nerve divides and goes to the medial head of the median as well as helping to form the lateral head (fig. 16). In one of these latter there is an additional branch to the medial head of the median nerve from the ventral division of the seventh cervical nerve, which gives off also from its dorsal division the lateral head of the ulnar nerve, and this also connects with the medial head of the median.

There is a ninth plexus in which the lateral head of the median nerve arises from the ventral branch of the intermediate trunk (seventh cervical nerve) of the plexus. The ventral branch of the cephalic trunk (fifth and sixth cervical nerves) does not con-

tribute to the formation of the median in the axilla but forms a large nerve which, after giving off three branches to the coracobrachialis muscle, divides into two branches. These pierce the coracobrachialis muscle separately, one becomes the musculocutaneous and the other joins the median nerve about the middle of the arm (fig. 17). The median nerve beyond this point may contain fibers from all the spinal nerves which contribute ordinarily to its formation. The lateral head of the ulnar in this case arises from the lateral head of the median (seventh cervical) and sends branches to the medial head of the median as well as to the ulnar nerve.

In 3 of the remaining 14 plexuses, the medial and lateral fasciculi of the plexus join to form a common stem that immediately splits up into median, ulnar and musculocutaneous nerves (fig. 27).

In two instances, the ulnar and median nerves arise from a common stem formed by the union of the lateral head of the median with the medial fasciculus of the plexus. Almost immediately after this stem is formed it divides into median and ulnar nerves (fig. 28). The above five cases have already been noted in discussing the ulnar nerve.

In 9 cases, the musculocutaneous nerve does not separate from the lateral fasciculus of the plexus, but the lateral fasciculus of the plexus joins the medial head of the median nerve to form a common stem. This passes down the arm and gives off the musculocutaneous nerve as a single definite nerve in 6 instances (fig. 6), but as a number of separate branches in 3 instances.

There were a number of other instances in which there were interesting interrelations between the median and the musculocutaneous nerves. Some of these will be considered in the description of the musculocutaneous nerve.

Of the 152 plexuses in which the median nerve arises by two heads in the usual way, 72, or 47.36 per cent neither receive nor give off any branches (figs. 5 and 7). But in the remaining cases one or the other of the heads of the median nerve either receive or give off branches.

The medial head of the median receives an additional branch

in 54 instances, or 35.52 per cent. In 10 cases this branch comes from the seventh cervical nerve, in 4 of them dividing and sending a subdivision to the ulnar nerve as the lateral head of the ulnar (fig. 10), and in 6 of them remaining undivided (fig. 19).

In 40 of the cases, the branch to the medial head of the median arises from the lateral fasciculus of the plexus. In 24 of these the branch divides into two parts sending one of the subdivisions to the ulnar nerve as the lateral head of the ulnar (figs. 1 and 3). In 1 of these instances it divides into three branches, one going to the ulnar, one to the lateral head of the median, and the third to the medial head of the median nerve, in one of these giving off in addition a branch to the coracobrachialis muscle (fig. 24). In another of the above instances, the lateral head of the ulnar, in addition to sending fibers to the ulnar and medial head of the median, gives off two branches that join the lateral head of the median. In a third case, in addition to a lateral head of the ulnar as above, there is an additional branch from the lateral fasciculus to the medial head of the median.

In 16 instances, the extra branch to the medial head of the median does not divide but goes to the medial head of the median only (fig. 20). In the 4 remaining instances, the branch to the medial head of the median nerve arises from the lateral head of the median, in 1 giving off the lateral head of the ulnar (fig. 8), and in 2 going to the medial head of the median only (fig. 21), and in 1 sending a branch to the lateral head as well as the medial head of the median nerve.

In only one instance is there a branch given off from the medial head of the median nerve. This branch goes to the coracobrachialis muscle (fig. 4), and may receive its fibers from the seventh cervical nerve since there is a branch from this to join the medial fasciculus of the plexus from which the medial head of the median nerve arises.

The lateral head of the median gives off a lateral branch or branches in 26 plexuses, 4 of these have already been noted where this branch goes to the medial head of the median. In 20 of the remaining 22 instances, the branch connects with the ulnar nerve as the lateral head of the ulnar, as in figure 2. In one

of these there is given off from the lateral head an additional branch that goes to the coracobrachialis muscle (fig. 22). In another instance the lateral head of the median gives off a branch which was said to be the lateral head of the ulnar but the nerve was broken and could not be verified. In the other case the branch supplies the coracobrachialis muscle.

The lateral head of the median nerve in five instances receives a branch. In one of these it is from the seventh cervical nerve (fig. 23). As already noted above, this branch comes from the lateral fasciculus of the plexus in two instances. In one case, the lateral head of the median receives a branch from the eighth cervical nerve (fig. 25), and in another from the medial fasciculus of the plexus (fig. 26).

From the above it will be noted that the lateral head of the median gives off branches in 26 instances but that it receives them in only 5; the medial head of the median gives off a branch in but one instance while it receives branches in 54. The tendency is therefore, in the great majority of cases, for the branches here to pass from the lateral toward the medial side of the plexus.

The median nerve received fibers from the fifth, sixth, seventh and eighth cervical and first thoracic nerves in 116 of the 136 cases reported by Wichmann from the literature. Schumacher has added 7 cases in which the above five nerves sent fibers to the median. In other words, if we disregard the fourth cervical nerve, which is not accounted for by these authors, all of the nerves which form the brachial plexus contribute to the formation of the median nerve in 123 of the 146 cases listed by them. In the remaining 23 cases, the fifth cervical nerve failed to send a branch to the median in 10 instances in Wichmann's series and in 2 of Schumacher's. The first thoracic nerve failed to send a branch to the median in 7 of Wichmann's and 1 of Schumacher's cases. Wichmann reports 3 instances in which other cervical nerves were lacking in the median, in one case it was the fifth and sixth cervical nerves, in one case the sixth cervical nerve and in one the eighth cervical nerve.

In none of the 175 plexuses of my series, was it possible to be

sure from the dissection that any of the nerves which go to make up the brachial plexus did not enter the median nerve. I am, however, reasonably certain that all of them do not contribute in all instances but I have complete records of so few macerated plexuses that I hesitate to quote statistics, since in only 1 of them was there a nerve lacking, the fifth cervical in this case.

If we assume then that in the other instances of my series all of the nerves that make up the brachial plexus enter the median nerve, we have 174 records. When these are added to the 123 instances recorded by Wichmann and Schumacher, we have 297 instances in which the fifth, sixth, seventh and eighth cervical and first thoracic nerves send fibers to the median nerve, or 92.52 per cent of the 321 records.

THE MUSCULOCUTANEOUS NERVE

The musculocutaneous nerve is usually described as formed by the division of the lateral fasciculus of the brachial plexus into the lateral head of the median nerve and the musculocutaneous nerve.

Since the nerve or nerves to the coracobrachialis muscle so frequently arise from other sources than the musculocutaneous, I have described the branch or branches to this muscle separately, and I have regarded the musculocutaneous nerve as complete whether or not it gave off these branches.

Among the 175 plexuses of my series, the musculocutaneous nerve arises by division of the lateral fasciculus of the plexus into medial head of the median nerve and musculocutaneous nerve in 155 cases, or 88.57 per cent (fig. 1). In two of these the lateral fasciculus of the plexus receives a branch from the eighth cervical nerve. These are of the type C of plexus (fig. 28). In another case the lateral fasciculus receives a branch from the medial fasciculus of the plexus, type G of plexus (fig. 8). In these three cases we cannot be sure that the eighth cervical nerve does not send fibers to the musculocutaneous nerve and in the last instance the first thoracic may also. In one of the above 155 cases the musculocutaneous is not complete but the major portion of it arises from the lateral fasciculus and the

branch which supplies the biceps muscle comes from a loop in the first part of the median nerve. In another of the 155 cases the lateral fasciculus does not divide as usual but the whole of it forms the musculocutaneous nerve, the lateral head of the median nerve being derived from the seventh cervical. In the arm, the musculocutaneous nerve sends a branch to the median nerve in this case (fig. 17).

There are three plexuses, 1.71 per cent of the 175, in which the musculocutaneous nerve arises from a trunk formed by the union of the medial and lateral fasciculi of the plexus (fig. 27). This trunk divides into median, ulnar and musculocutaneous as previously noted in connection with these other nerves. There are 9 instances, 5.14 per cent of the 175, in which the lateral fasciculus of the plexus joins with the medial head of the median and from the trunk thus formed the musculocutaneous nerve arises as a single branch (if we ignore the branch to the coracobrachialis) in 6 (fig. 6), and in two or more branches in 3 cases. In the above 12 plexuses the eighth cervical and the first thoracic nerves may send fibers to the musculocutaneous nerve.

In 9 of the above cases in which the musculocutaneous arises from a stem common to it and to the median or median and ulnar, there are records for both sides of the body and in all of these the arrangement on the other side was normal. Testut ('84) found the musculocutaneous fused with the median in 6 instances out of 105.

There are 8 plexuses, 4.75 per cent of the 175, in which the musculocutaneous nerve arises from the ventral division of the cephalic trunk of the plexus (figs. 15 and 16). In these cases, the seventh cervical nerve can take no part in the formation of the musculocutaneous nerve unless the musculocutaneous receives fibers of the seventh through its branches in the arm from the median or some other nerve. My records are complete through the arm for only 3 of these cases and these show no branches from the median to the musculocutaneous nerve but on the other hand in one of the plexuses there is a branch from the musculocutaneous to the median nerve.

My records for the musculocutaneous nerve are complete

through the arm for only 75 plexuses, in 18 of these the musculocutaneous nerve gives off a branch to the median nerve. (In one of these instances the musculocutaneous arises from the cephalic trunk formed only by the fourth, fifth, and sixth cervical nerves). In one of the above instances, the musculocutaneous also receives a branch from the median nerve.

In addition to these, in one of the plexuses where the musculocutaneous nerve arises from the median nerve as described above, it gives off a branch which joins the median nerve further distad. This branch arises from the median nerve distal to the point where the musculocutaneous would normally pierce the coracobrachialis muscle which it does not pierce in this case.

Although these records show that in nearly a quarter of the plexuses the musculocutaneous nerve gives off a branch in the arm which joins the median nerve, I do not believe that such a relation exists as frequently as this, for undoubtedly some of the records were made because of the occurrence of this anomaly. On the other hand, in 100 plexuses of my series the records were not preserved for the arm so that I cannot be positive that there may not have been more cases where the communication existed. If there were no more instances among the 175 plexuses of my series, which is unlikely, the anomaly is found in this series in 10.28 per cent of the 175. In the 75 where the records are complete, it is found in 18 or in 24 per cent of the cases. The truth probably lies somewhere between these two extremes.

Testut ('84) found a branch from the musculocutaneous nerve to the median nerve in 38 instances out of 105 examined, or 36.19 per cent, which is considerably larger than in my series.

In 3 cases, or in 4 per cent of the 75 complete records, the median nerve in the arm sends a branch to the musculocutaneous nerve. In one of these, as already noted, the median also receives a branch from the musculocutaneous. Testut, found a branch from the median to the musculocutaneous nerve in only two out of 105, or in less than 2 per cent, while Villar ('88) found such a branch in 3 out of 37 cases, or in 8.10 per cent.

In my series of 175 cases, the musculocutaneous nerve may receive fibers from the fourth, fifth and sixth cervical nerves

4 times, 2.28 per cent of the cases; from the fifth and sixth 4 times, 2.28 per cent of the cases; from the fourth, fifth, sixth and seventh cervicals 98 times, 56 per cent of the cases; from the fifth, sixth and seventh 54 times, 30.85 per cent of the cases; from the fourth to the eighth cervical inclusive, 2 times, 1.14 per cent of the cases; from the fourth cervical to the first thoracic inclusive 6 times, 3.42 per cent of the cases; from the fifth cervical to the first thoracic 7 times, 4 per cent of the cases.

Herringham found the seventh entering the musculocutaneous in only 4 out of 39 cases, and in 28 examined to see if both the fifth and sixth entered he found the sixth absent in only one. Wichmann reports, from the literature, its origin from the fourth, fifth and sixth in 1 case, and from the fifth and seventh in another, from the fifth and sixth in 60 cases and from the fifth, sixth and seventh in 44 cases. Schumacher found the fifth and sixth entering 5 times, the fifth, sixth and seventh 4 times, and the fourth, fifth and sixth once.

If these 155 records are added to my 175 cases we have a total of 330 records of the musculocutaneous nerve. In 6 of these, or 1.82 per cent, it may receive fibers from the fourth, fifth and sixth cervical nerves. In 103, or 31.21 per cent, its fibers may come from the fifth and sixth. In 204, or 61.82 per cent, the seventh also sends fibers to the musculocutaneous, which in 98 of these, or 29.70 per cent is formed by the fourth, fifth, sixth and seventh, and in 106, or 32.12 per cent, by the fifth, sixth and seventh. In two its fibers come from the fourth to the eighth cervical nerves inclusive; in 6 or 1.82 per cent, from the fourth cervical to the first thoracic inclusive and in 7, or 2.12 per cent, from the fifth cervical to the first thoracic inclusive. In 1 instance it is formed from the fifth and seventh cervical nerves and in 1 from the fifth cervical alone. The seventh cervical nerve appears to take part in the formation of the musculocutaneous nerve in 220 instances, or more than two-thirds of the cases. In nearly a third of the cases, however, the seventh cervical sends no fibers to the musculocutaneous and it is not at all impossible that maceration with acid would show it to be absent more often.

THE NERVE TO THE CORACOBRACHIALIS MUSCLE

In the above description of the musculocutaneous nerve no mention has been made of any of its branches of distribution. The nerve to the coracobrachialis muscle is usually described as a branch of the musculocutaneous nerve. In many cases, however, it takes origin from the brachial plexus independent of the musculocutaneous nerve and there may be more than one nerve to the muscle.

A record of the branches of the musculocutaneous nerve was not made for all of the plexuses of my series, so that I have but 109 records of the nerve to the coracobrachialis muscle.

In 54 plexuses, or 49.54 per cent of the 109 in which there are records, the nerve to the coracobrachialis muscle arises as a branch of the musculocutaneous nerve. There is but a single branch arising from the musculocutaneous nerve for the muscle in 30 of these cases (fig. 2 and 15). In 2 of the above the musculocutaneous arises from the ventral division of the cephalic trunk. In the remaining 24 instances there is more than 1 branch. In 14 of these, there are two separate branches (fig. 25), in 6 of them there are 3 independent branches (figs. 3 and 14); and in 3 instances there are 4 branches. In one of these last three cases, the musculocutaneous nerve arises from the ventral branch of the cephalic trunk of the plexus, but one of its branches to the coracobrachialis muscle receives shortly after its origin a small branch from the ventral division of the seventh cervical nerve (fig. 18). In the remaining instance in which the nerve to the coracobrachialis arises from the musculocutaneous nerve, the nerve receives soon after its origin a small branch from the medial fasciculus of the plexus and in this case there is a second nerve to the muscle coming from a branch connecting the musculocutaneous with the median nerve.

In the remaining 55 plexuses at least one of the nerves to the coracobrachialis muscle arises from some other branch or division of the brachial plexus than the musculocutaneous nerve.

In 35 instances, or 32.11 per cent of the 109 plexuses, one of the branches to the coracobrachialis muscle arises from the lateral fasciculus of the plexus. In 19 of these, this is the only

branch to the muscle (figs. 8, 10, 13). In another of the above 19 the nerve to the coracobrachialis muscle gives off a branch that rejoins the lateral fasciculus close to the point where the musculocutaneous nerve arises. In one of the above 19 the musculocutaneous arises from a trunk in common with the ulnar and median nerves and in another it is given off from the median in two separate branches.

In 3 instances there is a second branch from the lateral fasciculus to the coracobrachialis muscle. In 8 cases the second branch to the muscle arises from the musculocutaneous nerve (fig. 12), and in 5 others there are two additional branches both coming from the musculocutaneous nerve.

There is another plexus in which in addition to the nerve from the lateral fasciculus there is another nerve to the muscle arising from the seventh cervical nerve and another in which the second nerve comes from the lateral head of the ulnar nerve (fig. 24).

In 8 cases the nerve to the coracobrachialis muscle arises from the seventh cervical nerve. (These are in addition to the case mentioned above where there was a communicating branch from the seventh cervical to one of the four branches arising from the musculocutaneous nerve.) In 4 of these this is the only branch to the muscle, in 4 there is a second branch coming from the musculocutaneous nerve.

In 4 plexuses in which the musculocutaneous does not arise from the lateral fasciculus of the plexus but from the trunk formed by the lateral fasciculus and the medial head of the median, the nerve to the coracobrachialis also arises from this trunk (fig. 6). In one case one of the nerves to the muscle arises from the median, the other from the musculocutaneous which in this case also comes from the median. In 2 instances one nerve comes from the lateral head of the median and the other from the musculocutaneous (fig. 22). In 1 case there is but one branch and this comes from the lateral head of the median, in another the single branch to the muscle comes from the medial head of the median (fig. 4).

My maceration experiments seem to indicate that in some instances where at least one of the nerves to the coracobrachialis

muscle appears to arise from the lateral fasciculus of the plexus or from the musculocutaneous nerve, the fibers may be found to come from the seventh cervical nerve.

In my series of 109 records of the nerve to the musculocutaneous it was represented by a single nerve in 61, or 55.45 per cent of the 109 instances. The muscle is supplied by 2 nerves in 34, or 31.19 per cent; by 3 nerves in 11 cases, or 10.09 per cent; and by 4 nerves in only 3 instances or 2.57 per cent. It is probable that in some cases one or more of the nerves to the muscle may have been broken in the dissection or overlooked in the verification. It is altogether probable that in some cases at least there was an additional nerve that was not exposed since the foramen in the coracobrachialis muscle through which the musculocutaneous nerve passes was not opened up until late in the dissection and one of the branches to the muscle is often given off by the musculocutaneous nerve during its passage through the muscle.

The nerve to the coracobrachialis in my series arises in such a way that fibers of only the fifth and sixth nerves might enter it in two instances and in only 4 instances does it arise in such a way that the fibers of all the nerves but the seventh cervical can be excluded. In 61 instances its origin is such that the fibers of the fourth to the seventh cervical nerves inclusive might enter and in 31 cases fibers from the fifth to the seventh. In 11 plexuses fibers of the nerves caudal to the seventh cervical could not be excluded. In 6 of these the fourth to the eighth cervical and the first thoracic; in 4, the fifth to the eighth cervical and the first thoracic; and in 1 the fourth to the eighth cervical might have contributed.

Herringham found the nerve received its fibers from the seventh cervical nerve only, in all cases, except one in which there were fibers from the sixth cervical also.

THE SUPRASCAPULAR NERVE

The suprascapular nerve is usually described as arising from the cephalic trunk of the brachial plexus formed by the junction of the fifth and sixth cervical nerves.

In my series there are 172 plexuses in which the records are satisfactory for the suprascapular nerve. In 108 or 62.79 per cent of these it arises from the cephalic trunk of the plexus. This trunk is formed by the union of the fourth, fifth and sixth cervical nerves in 69 (fig. 6), and by the fifth and sixth in 39 (fig. 8). In 12 instances the suprascapular nerve arises from the ventral branch of the cephalic trunk (fig. 12), and in only 1 of these the fourth cervical nerve fails to take part in the formation of this trunk. In 22 instances the nerve arises from the dorsal branch of the cephalic trunk and in 9 of these the fourth cervical does not enter into the formation of the brachial plexus (figs. 3 and 7).

In many cases it is very difficult to tell whether the suprascapular nerve arises from the cephalic trunk or from the dorsal or ventral branch of it since it takes its origin just at the point where the trunk divides into dorsal and ventral branches (fig. 11). In all cases I have considered it as arising from the trunk unless it could be clearly shown that all of its fibers came from either the dorsal or the ventral branch of the trunk.

In 7 of the above 108 cases, the suprascapular nerve arises from a common stem which in 5 divides into suprascapular and subscapular nerves (fig. 5), and in one the stem divides into the suprascapular nerve and a branch which joins the subscapular nerve, while in the other case it arises from a similar common stem which divides into the suprascapular nerve and the dorsal scapular nerve to the rhomboid muscles.

All 7 of the above exceptional cases belong to group 1, types A and B of plexus. In 3 of the instances the branch arises from the cephalic trunk; in 2 from the ventral branch of this trunk; and in 2 from the dorsal branch of it.

In 13 of the 172 plexuses studied the suprascapular nerve arises from the trunk formed by the union of the fourth and fifth cervical nerves (fig. 13). In one other case it comes from the ventral branch of the above trunk and in another from the dorsal branch of the trunk. In 14 instances it arises from the fifth cervical nerve alone (fig. 2). In one of these latter the fourth cervical nerve enters the plexus but the suprascapular arises high up in

the plexus before the fourth nerve joins the fifth. In one other case the suprascapular arises from the ventral division of the fifth cervical nerve (fig. 15).

From the above it will be seen that in 30 plexuses out of 172 it can be surely determined that no nerve caudal to the fifth cervical enters the plexus. In 15 of these, the fourth nerve may contribute but in the remaining 15 plexuses the suprascapular comes exclusively from the fifth cervical nerve.

There are 142 cases from which the sixth cervical nerve can not be excluded from the suprascapular nerve. In 93 of them the suprascapular may receive fibers from the fourth cervical nerve (group 1 plexuses) and in 49 can receive no such fibers (groups 2 and 3 plexuses). It will be seen then that while the plexuses in which the suprascapular nerve receives no fibers from the sixth cervical nerve are almost equally distributed between those with and those without a branch from the fourth cervical nerve; that the plexuses in which the sixth cervical nerve, that is, a more caudal nerve, may contribute to the suprascapular nerve are nearly twice as many of them in the group with a branch from the fourth cervical as in the group without this nerve.

If the plexuses of group 1 are more cephalic in position we should expect to find fewer instances among them in which the suprascapular nerve receives fibers from the sixth cervical nerve than in the more caudally placed plexuses of groups 2 and 3, while the reverse appears to be the case. It must, of course, be remembered that some of the plexuses in which fibers from the sixth nerve could not, by dissection, be excluded from the suprascapular nerve would show, if macerated in nitric acid, that fibers from this nerve did not enter into the formation of the suprascapular.

Wichmann reports upon only 34 cases of suprascapular nerve from the literature, in 17 of these it arose from the fifth and sixth cervical and in 17 from the fifth cervical nerve alone. Schumacher gives the fifth cervical as the dominant spinal nerve supplying fibers to the suprascapular but he also notes in his table the presence of fibers from the sixth and occasionally from

the fourth cervical nerve. He does not, however, give the number of cases of each that he found.

THE SUBCLAVIUS NERVE

The nerve to the subclavius muscle is usually described as arising from the cephalic trunk of the brachial plexus, occasionally from the fifth cervical nerve.

The nerve is a slender twig and is often broken in the dissection so that among the 175 plexuses of my series, it was satisfactorily dissected and recorded in only 83 cases.

In 18 or 21.68 per cent of these it arises from the fifth cervical nerve after the fourth has joined it so that the fibers of the fourth cannot be excluded. In 13 instances it is found as a single separate branch (fig. 11); in 3 it arises from a common stem with a branch to the phrenic nerve; and in 2 from a common stem with the dorsal scapular nerve to the rhomboid muscle.

In 22 plexuses or 26.50 per cent it arises from the fifth cervical nerve only; in 10 as a separate branch (fig. 16), and in 10 from a common stem with a branch to the phrenic nerve (fig. 25); and in 1 from a branch common to it and the dorsal scapular nerve. There is also one of the above instances in which it arises from a stem common to it and a branch to the phrenic nerve that arises from the fifth cervical nerve but receives a branch from the sixth before dividing (fig. 7).

In 41 or 49.39 per cent of the 83 cases it arises from the cephalic trunk or its ventral branch. In 27 of these this trunk is formed by the fourth, fifth and sixth cervical nerves. In 20 of these instances it comes from the main branch of the cephalic trunk; 14 times as a single branch (fig. 6), and 6 times in combination with a branch to the phrenic nerve. In 7 of the above 27 cases the subclavian nerve arises from the ventral branch of the cephalic trunk, in 5 as a single branch, in 1 in combination with a branch to the phrenic nerve and in 1 with the lateral anterior thoracic nerve.

In 14 of the 41 instances, in which the nerve arises from the cephalic trunk, this trunk is formed by the fifth and sixth cervical nerves only. In 9 of these the nerve arises from the undivided

cephalic trunk; in 6 instances singly (fig. 8); and in 3 in combination with a branch to the phrenic nerve. In 5 of the 14 instances it arises from the ventral branch of the cephalic trunk, in 4 singly and in 1 with the lateral anterior thoracic nerve.

In 1 instance the nerve to the subclavius arises as a single branch from the sixth cervical nerve and in 1 instance it arises also as a single branch from the lateral cord of the plexus. The cord in this latter instance is formed from the ventral branches of the cephalic trunk, formed from the fourth, fifth and sixth cervical nerves, combined with the ventral branch of the seventh cervical nerve.

In 54 of the above plexuses or 65.06 per cent the subclavius nerve arises as a single branch. In 24 or 28.67 per cent it comes from a stem common to it and a branch to the phrenic, in 3 from a branch common to it and the dorsal scapular nerve, and in 2 from a stem from which one of the branches of the lateral anterior thoracic nerve also arises.

The records given by others of the spinal nerve or nerves from which the subclavius nerve arises are very incomplete, doubtless because the nerve is so delicate and so often broken in the dissection. Wichmann in his synopsis of the literature up to 1900 does not report clearly the number of cases recorded. He states that it has been found arising from the third cervical nerve by one author, from the fourth by one, and from the fifth by five authors. The nerve was reported as coming from the fifth and sixth cervical nerves by two authors; from the fifth, sixth and seventh by 1. The sixth cervical alone gave rise to the nerve in 2 instances reported by one author and the seventh and eighth by 1. He gives the fifth and sixth as the normal of Renz. Schumacher reports the fourth and fifth as found in Bolk's case. In his own investigations the fifth was the dominant nerve; the sixth sometimes entering. My results show that out of 83 satisfactory records the nerve to the subclavius may receive fibers from the fourth and fifth cervical nerve in 18 instances; from the fifth cervical only in 21 cases; from the fourth, fifth and sixth cervical nerves in 27 instances; from the fifth and sixth cervical nerve in 15; from the sixth cervical in 1; from the fourth,

fifth, sixth, seventh in 1. It will be noted then that in 82 instances or in every plexus but one, the fifth cervical may send fibers to the subclavius; that the fourth cervical may enter in 47; the sixth in 44; and the seventh but once. In 39 instances the fibers may come from the fifth or fourth and fifth cervical nerves where the fourth enters the plexus, and in 42 cases the fibers may come from the fifth and sixth, or the fourth, fifth and sixth cervical nerves where the fourth enters the plexus.

THE CUTANEOUS NERVES TO THE MEDIAL SIDE OF THE ARM

The cutaneous nerves to the axillary fossa and its borders and the nerves to the skin of the medial side of the arm and the contiguous regions on the dorsomedial and ventromedial side of the arm are derived from several different sources. The nerves to the axillary region are mostly derived from the lateral cutaneous branches of the intercostal nerves, usually the second, and third, but at times the first and fourth contributing and also branches from the medial brachial cutaneous and the medial antibrachial cutaneous nerves or a separate nerve from the plexus arising independently. The medial side of the arm distal to the axilla and the adjoining dorsal and ventral regions is supplied mostly by the medial brachial cutaneous and intercostobrachial nerves. This latter coming usually from the second intercostal nerve occasionally from one of the others. There are also at times branches to this region of the arm derived from the medial antibrachial cutaneous nerve or an independent nerve that perhaps should be called a second medial brachial cutaneous. In addition, there are branches to the adjoining dorsal region derived from the axillary and radial nerves, with these latter I shall not deal now. It has been extremely difficult to get satisfactory student records of the distribution of the nerves of this region, even when the dissections were most excellent and the peripheral attachments of the nerves were not disturbed. The nerves here, however, were so frequently loosened that their exact distribution was difficult to verify. For the above reasons it was often difficult when there were several branches from the plexus to determine definitely how they should be named. In

these cases the nerves were classified and named as carefully as possible at the time the record was verified but as the dissection of the plexus came much later than the working out of the peripheral distribution, the exact area supplied by each branch from the plexus could not always be determined.

THE MEDIAL BRACHIAL CUTANEOUS NERVE

The medial brachial cutaneous nerve (lesser internal cutaneous or nerve of Wrisberg) is usually described as rising from the medial trunk of the brachial plexus. My records are complete and satisfactory for 166 plexuses. In 137, or in 82.59 per cent, it is represented by a single branch and in 28 plexuses it is given off as two branches and in 1 as three separate filaments. In addition to this there are 9 cases in which there is recorded a branch given off by the medial antibrachial cutaneous nerve near its origin that anastomoses with the intercostobrachial. I am inclined now to classify all these as accessory medial brachial cutaneous nerves.

As this nerve and its branches are very small and as they are imbedded in a considerable mass of connective tissue it must be remembered that there is great danger of breaking or entirely overlooking and cutting away small accessory and communicating branches. This report is then positive for the branches and connections found and recorded, but it is not negative for those not recorded. There were undoubtedly other cases in which there was more than one nerve which supplied the region usually assigned to the medial brachial cutaneous nerve. There is much need of more very careful study of the interrelations of this nerve, the intercostobrachial, the posterior brachial cutaneous branch of the radial, and the cutaneous branches from the medial antibrachial cutaneous nerve which supply the medial and dorsal surfaces of the arm.

Of the 137 specimens in which the nerve arises as a single branch it comes directly and separately from the caudal trunk or medial fasciculus of the plexus in 92 (fig. 3) and from the dorsal division of the caudal trunk in 2 (fig. 7). In 11 other cases it arises separately, in 6 from the first thoracic nerve, in 4 from the ulnar

nerve and in 1 by two roots, one from the eighth cervical and one from the first thoracic nerve.

In 32 instances the medial brachial cutaneous arises from a common branch from which the medial antibrachial cutaneous or the medial anterior thoracic or both of these arise (figs. 6, and 17). In 31 of these, this branch arises from the caudal trunk or the medial fasciculus of the plexus and in the other case from the first thoracic nerve.

In 29 cases there is more than one nerve that was classed as medial brachial cutaneous, in addition to the cases where a branch from the medial antibrachial cutaneous anastomoses with the intercostobrachial.

There are 36 of the 166 satisfactory plexuses in which a single medial brachial cutaneous nerve arises directly from the caudal trunk of the plexus (fig. 3). In 23 of these there is an anastomoses with the intercostobrachial. One of these sends a branch to the medial anterior thoracic nerve, another to the medial antibrachial cutaneous and a third to the radial, while two others receive branches from the medial antibrachial cutaneous nerve. In 13 cases there is no record of communication between the medial brachial cutaneous and the intercostobrachial. In two of these the medial brachial cutaneous receives a branch from the medial antibrachial cutaneous and in 2 others there is an anastomosis between a branch of the medial antibrachial cutaneous and the intercostobrachial nerve.

There are 6 instances in which the medial brachial cutaneous nerve is single and arises from the caudal trunk of the plexus in combination with some other nerve. In 2 of these it is combined with the medial anterior thoracic nerve and in 1 of these it sends a branch to the medial antibrachial cutaneous which anastomoses with the intercostobrachial. In 3 cases it is combined with the medial antibrachial cutaneous, in 2 of these anastomosing with the intercostobrachial. In the last of the 6 cases it arises from a stem common to it and both of the above mentioned nerves and anastomoses with the intercostobrachial (fig. 27).

In 56 plexuses the single medial brachial cutaneous nerve arises directly from the medial fasciculus of the plexus. In 23 of these

there is no anastomosis with the intercostobrachial (fig. 21). One of the 23 sends a branch to the medial antibrachial cutaneous and in 2 others there is an anastomosing branch from the medial antibrachial cutaneous to the intercostobrachial nerve. In the remaining 33 cases where the nerve arises directly it anastomoses with the intercostobrachial (fig. 18). In one of these it arises by 2 heads (fig. 8), in another it sends a branch to the posterior division of the caudal trunk. In 3 of the above, the medial antibrachial cutaneous also anastomoses with the intercostobrachial nerve.

There are 25 instances in which the medial brachial cutaneous nerve arises from the medial fasciculus of the plexus in combination with some other nerve. In 16 of these, it is in common with the medial antibrachial cutaneous (fig. 15) and in 8 of these it anastomoses with the intercostobrachial In 3 of the above 25 cases, the medial brachial cutaneous is combined with the medial anterior thoracic nerve (fig. 14). In 6 instances it arises from a branch common to it and the medial antibrachial cutaneous and the medial anterior thoracic nerve (fig. 17), and in 3 of these it anastomoses with the intercostobrachial nerve.

There are 2 instances in which the medial brachial cutaneous nerve arises directly from the dorsal division of the caudal trunk and in one of these it anastomoses with the intercostobrachial nerve (fig. 7).

There are 4 plexuses in which it arises singly from the ulnar nerve and in one of these it anastomoses with the intercostobrachial.

In 6 instances there is a single medial brachial cutaneous nerve arising directly from the first thoracic nerve and in 5 of these it anastomoses with the intercostobrachial. In one case it arises from the first thoracic by a common stem with the medial antibrachial cutaneous nerve and in another sends a branch to the dorsal division of the caudal trunk.

There are 29 cases in which there are 2 or more branches arising from the brachial plexus that were classed as medial brachial cutaneous nerves. This does not necessarily mean that there is a larger supply in these cases but that the branches arise from

the plexus separately instead of by a common stem. In 6 of the above both branches arise from the caudal trunk and in 5 of them one branch anastomoses with the intercostobrachial. In one of them, one branch receives a branch from the medial antibrachial cutaneous (fig. 10); in another case a branch of one of the nerves joins the medial antibrachial cutaneous (fig. 5); and in a third plexus one branch communicates with the radial nerve. In one of these plexuses one of the branches arises in common with the medial anterior thoracic nerve. In 8 other cases where there are 2 branches, one of them arises from the caudal trunk and this branch anastomoses with the intercostobrachial nerve. It arises in common with the medial anterior thoracic in 2 instances. In 7 of the above the second branch arises from the medial fasciculus of the plexus and this does not anastomose in 5 but communicates with the intercostobrachial in 2 instances and also with the medial antibrachial cutaneous in one of them. Another of the above sends a branch to the posterior fasciculus of the plexus. In one of the above 7 cases the nerve arises from a stem common to it and the medial anterior thoracic (fig. 11); another from a stem in common with the medial antibrachial cutaneous nerve.

In 12 cases both of the branches arise from the medial fasciculus. In 7 of these they both arise directly from the medial fasciculus and in 4 neither branch anastomoses but in 2 one branch and in one both branches anastomose with the intercostobrachial nerve. Of the remaining 5 cases, in 4 one branch arises directly from the medial fasciculus and anastomoses with the intercostobrachial but the second branch arises from the medial fasciculus in common with the medial antibrachial cutaneous in one (fig. 20), with the medial anterior thoracic in one, with both of these nerves in one. In the fourth case both branches arise from the medial fasciculus of the plexus; one with the medial antibrachial cutaneous; one with the medial anterior thoracic. In the fifth instance, both branches arise from the medial fasciculus by a common stem with the medial antibrachial cutaneous.

In one instance one branch is from the medial fasciculus and

the other comes from a small dorsal division of the caudal trunk that joins the posterior fasciculus. Both anastomose with the intercostobrachial. In one case both branches arise from the first thoracic nerve and one of them anastomoses with the intercostobrachial. There is one instance where there are 3 branches that were classified as medial brachial cutaneous nerves; these all arise from the medial fasciculus of the plexus from a stem from which the medial antibrachial cutaneous also arises. One or two of them might possibly be classified as branches of this latter nerve.

The medial brachial cutaneous nerve arises by itself from one of the main branches of the brachial plexus in 103 of the 137 plexuses where there was a single nerve.

In 20 instances the single medial brachial cutaneous arises from a stem common to it and the medial antibrachial cutaneous; in 5 from a stem common to it and the medial anterior thoracic; and in 8 from a stem common to it and both of the above nerves. In the 29 cases where there are two or more nerves, there are 59 nerves recorded and 42 of these arise separately; 10 with the medial antibrachial cutaneous; 6 with the medial anterior thoracic; and one with both of these nerves. It will be seen then that the medial brachial cutaneous arises from the plexus as an independent branch in 73.97 per cent of the cases.

The records show that the medial brachial cutaneous nerve anastomoses with the intercostobrachial in 101 instances or in 60.84 per cent of the cases. There is no doubt that there were many other plexuses in which such a connection existed but it was broken before the record could be verified.

The medial brachial cutaneous nerve arises from the caudal trunk of the brachial plexus or one of the divisions of this in 157 of the 166 plexuses. It therefore may receive fibers from the eighth cervical and first thoracic in all of these. In addition, there is one case where it arises from these nerves by separate roots. The eighth cervical and first thoracic nerves may send fibers to it in 95.18 per cent of the cases. In one of the type A plexuses it arises from the caudal trunk distal to the point where the branch from the seventh cervical joins this trunk so that in this case the seventh cervical cannot be excluded.

In Wichmann's tabulation, the eighth cervical and first thoracic are credited with sending fibers to the nerve in 9 cases, the first thoracic in 84 and the seventh cervical in 1.

THE MEDIAL ANTIBRACHIAL CUTANEOUS NERVE

The medial antibrachial cutaneous (internal cutaneous, ulnar antibrachial cutaneous) nerve is usually described as arising from the caudal trunk of the brachial plexus.

There are 174 satisfactory records in my series. There are 95 of these in which the nerve arises from the medial fasciculus of the plexus singly and with no anastomosis with the intercostobrachial. In 86 of these there are no anastomoses with any nerve (fig. 4); in 3 the nerve receives a branch from the medial anterior thoracic nerve; in one it receives a branch from the caudal trunk, a sort of second head; in 2 a branch from the medial brachial cutaneous joins it and in 4 it sends a branch to this nerve. There are 10 other plexuses in which the medial antibrachial cutaneous nerve arises from the medial fasciculus of the plexus but it anastomoses with the intercostobrachial in these, and in one of them it receives a branch from the medial brachial cutaneous nerve.

There are 36 cases in which the medial antibrachial cutaneous nerve arises from the medial fasciculus of the plexus from a stem common to it and some other nerve. In 19 cases it arises with the medial brachial cutaneous (fig. 6); in 8 with the medial anterior thoracic; and in 7 with both of these nerves (fig. 1). In 17 of these cases there is no anastomosis with the intercostobrachial but in the 2 other cases there is. In one of these it arises with the medial brachial cutaneous and sends a branch to the radial; in the other, with the medial anterior thoracic and receives a branch from the medial brachial cutaneous.

The medial antibrachial cutaneous nerve arises from the caudal trunk of the plexus singly and without anastomosing with the intercostobrachial in 12 instances (fig. 2). In one of these there are 2 roots; in one it received a branch from the medial brachial cutaneous; and in one it gives a branch to the radial nerve. There are 4 other cases in which the nerve arises from

the caudal trunk of the plexus and has no anastomosis with the intercostobrachial. In 2 of these it arises in combination with the medial brachial cutaneous; in one with the medial anterior thoracic nerve; and in one with both of these (fig. 27).

There are 7 cases in which the medial antibrachial cutaneous nerve arises from the ulnar nerve and has no anastomosis with the intercostobrachial. In one of these it arises from a stem common to it and the medial anterior thoracic.

There are 4 instances where the nerve arises from the first thoracic without connection with the intercostobrachial. In one of these it arises in common with the medial brachial cutaneous nerve.

There are 6 plexuses in which 2 nerves were recorded as medial antibrachial cutaneous. In 2 these both arise from the medial fasciculus of the plexus, and in one of these one nerve anastomoses with the intercostobrachial and the other arises from a stem in common with the medial brachial cutaneous; in the third case, both nerves come from the caudal trunk; in the fourth both come from the medial fasciculus, the first by a common stem with the medial brachial cutaneous and the second singly. In the fifth instance with two nerves, one arises from the medial fasciculus and the other a slender fascicle from the eighth cervical nerve. In the sixth instance one comes from the caudal trunk and one from the medial fasciculus of the plexus.

There are 143 or 82.18 per cent of the plexuses in which the medial antibrachial cutaneous nerve arises from the medial fasciculus of the plexus besides 3 in which there are 2 nerves. There are 16 plexuses in which the nerve arises from the caudal trunk besides 2 in which the nerve is double.

In all the plexuses except 4 in which the nerve arises from the first thoracic nerve only, we cannot exclude fibers of the eighth cervical as well as the first thoracic. There are 170 such cases or 97.70 per cent. In 3 of the 5 plexuses of type A that are included in the above, the seventh cervical nerve also cannot be excluded. The medial antibrachial cutaneous nerve may receive its fibers from the eighth cervical and first thoracic in 167 cases; from the seventh and eighth cervical and first thoracic

in 3; and from the first thoracic in 4. It must always be remembered that the second thoracic is not considered in my records.

Wichmann reports from the literature 51 cases in which the eighth cervical and first thoracic nerves entered the medial antibrachial cutaneous and 38 in which the first thoracic only enters the nerve. Schumacher found out of 10 cases that the eighth cervical and first thoracic enter the nerve in 8 and the first thoracic in 2. Adding the above to my records, we have the possibility of the medial antibrachial cutaneous nerve deriving its fibers from the eighth cervical and first thoracic in 226 cases; the first thoracic alone in 44 cases and the seventh and eighth cervical and first thoracic in 3.

In 25 instances the medial antibrachial cutaneous nerve arises from a common stem with the medial brachial cutaneous; in 9 with the medial anterior thoracic and in 9 with both of the above nerves. In 10 instances it has a connection with the intercostobrachial. In one of these the nerve is double and both branches communicate.

THE MEDIAL ANTERIOR THORACIC NERVE

The medial (internal) anterior thoracic nerve is usually described as arising from the caudal trunk of the brachial plexus.

In my series there are 151 satisfactory records for this nerve. In 105 of these, or 69.53 per cent, it arises from the medical fasciculus of the plexus (fig. 3); in 38, or 24.50 per cent, it arises from the caudal trunk of the plexus (fig. 2); in 3 from the eighth cervical nerve (fig. 4); in one from the medial head of the median nerve; and in one from the ulnar nerve; in 3 cases by two roots, one from the medial fasciculus and one from the seventh cervical nerve.

Of the 105 cases that arise from the medial fasciculus of the plexus, in 83 the nerve arises directly from the plexus, in one case by two roots. In 9 of the 83 the nerve gives off a branch and in one it receives a branch from the medial brachial cutaneous nerve. In 4 instances the branch given off goes to the skin of the arm, shoulder or axilla. In one it supplies an axillary slip of the latissimus dorsi muscle. In this case the seventh cervical nerve

sends a branch to the caudal trunk, plexus type A, so that this nerve cannot be excluded from the medial anterior thoracic. In 3 cases it communicates with the medial antibrachial cutaneous nerve and in one with the intercostobrachial.

In the other 22 instances where the nerve arises from the medial fasciculus of the plexus it comes off from a stem common to it and some other nerve, with the medial antibrachial cutaneous in 8 instances; with the medial brachial cutaneous in 6, and with both of these in 8 (fig. 1).

In 32 of the 38 instances in which the medial anterior thoracic nerve arises from the caudal trunk of the plexus, it is single and in 6 it is from a stem, with the medial brachial cutaneous in 5 (fig. 11), and with both the medial brachial cutaneous and medial antibrachial cutaneous in one (fig. 27).

The eighth cervical and first thoracic nerves could neither of them be excluded from the medial anterior thoracic nerve in 144 of the 151 satisfactory records, or 95.36 per cent. The seventh and eighth cervical and first thoracic nerves may send fibers in 4 cases, while the nerve receives fibers only from the eighth in 3 instances.

Herringham reports the medial anterior thoracic nerve as receiving its fibers from the eighth cervical and first thoracic nerves in 8 out of 10 cases. In the other two the eighth cervical alone sent fibers to it. I found only 3 cases out of 151 in which the first thoracic nerve failed to enter the plexus. It is possible that some others if macerated would show that both nerves did not enter into its formation, but this could not be proven by dissection. In 3 cases the seventh cervical nerve positively entered the nerve and in the plexus of type A it may have entered.

THE LATERAL ANTERIOR THORACIC NERVE

The lateral (external) anterior thoracic nerve is usually described as arising from the cephalic trunk of the plexus.

In my series there are 166 satisfactory cases. The nerve arises by a single root in 39 instances, or in 23.49 per cent of the cases; by two roots in 91, or 54.81 per cent; by three roots in 33, or in 19.87 per cent; and by four roots in 3.

Of the 39 plexuses in which it arises by a single root, this comes from the cephalic trunk of the plexus in 2 (fig. 2), from the ventral division of the cephalic trunk in 12 (fig. 4), from the lateral fasciculus in 17 (fig. 21), from the seventh cervical nerve in 2, and from the ventral division of the seventh cervical nerve in 6. One of the nerves that arises from the lateral fasciculus sends a branch to the medial head of the median nerve.

In 73, or 78.02 per cent, of the 91 cases where the nerve arises by two roots, one of these comes from the ventral division of the cephalic trunk of the plexus and the second comes from the ventral division of the seventh cervical nerve (fig. 3). In 7 cases both roots arise from the lateral fasciculus of the plexus (fig. 20). One comes from the ventral division of the cephalic trunk and one from the lateral fasciculus in 3 (fig. 11); one from the ventral division of the sixth and one from the ventral division of the seventh cervical nerve in 2. There is one instance of each of the following: one branch from the ventral and one from the dorsal division of the cephalic trunk, one from the lateral fasciculus of the plexus and one from the seventh cervical nerve, both from the seventh cervical, one from the seventh and one from the dorsal division of the sixth cervical, one from the lateral fasciculus of the plexus and one from the lateral head of the ulnar, one from the lateral fasciculus and one from the combined cephalic and intermediate trunks.

In 18, or 54.54 per cent, of the 33 plexuses where there are three roots to the lateral anterior thoracic nerve, one of these arises from the ventral division of the cephalic trunk and the other two from the ventral division of the intermediate trunk (seventh cervical nerve) (fig. 1). In 5 cases one root comes from the ventral division of the cephalic trunk, one from the lateral fasciculus of the plexus and one from the seventh cervical nerve. In 4 cases two roots come from the ventral division of the cephalic trunk and one from the seventh cervical nerve, or its ventral division. In 2 cases all three roots come from the lateral fasciculus of the plexus. Each of the following arrangements is found in one plexus only: one root from the ventral division of the fifth, one from the ventral division of the sixth and one from the ventral

division of the seventh cervical nerves; one root from the fifth, one from the ventral division of the cephalic trunk and one from the ventral division of the seventh cervical nerve; one root from the ventral division of the cephalic trunk, one from the ventral division of the seventh and one from a ventral branch from the eighth cervical that goes to the lateral fasciculus (fig. 27); one root from the lateral fasciculus of the plexus and two from the ventral division of the seventh cervical nerve.

The three plexuses in which there are four roots have the same arrangement, one root comes from the ventral division of the cephalic trunk, one from the lateral fasciculus and two roots from the ventral division of the seventh cervical nerve. In one of these the root that comes from the cephalic trunk gives off a branch to the phrenic nerve.

In the total of 166 records there are only 15 cases in which the seventh cervical nerve may not contribute to the lateral anterior thoracic nerve and as 14 of these are in the group where there is but a single root to the nerve it makes one suspicious that in spite of all our care a second root may have been broken or overlooked. These 15 cases are nearly equally distributed between the group 1 and group 2 of plexuses, 8 of group 1 and 7 of group 2. There are 87 cases, or 52.40 per cent, in which the fourth, fifth, sixth and seventh cervical nerves may send fibers to the lateral anterior thoracic, and 51, or 30.72 per cent, in which the fifth, sixth and seventh may contribute. There is one plexus in which the fourth, fifth, sixth, seventh and eighth cervical nerves can none of them be excluded. This is the only instance in which fibers may come from the eighth. The sixth and seventh are the only contributing nerves in 3 instances and the seventh alone in 9.

Herringham believed that the seventh cervical nerve always contributes to the lateral anterior thoracic. He reports on 13 dissections that the fifth and sixth cervical nerves also contribute to it in 5 instances and the sixth only in 8 cases. I can be sure that the fifth cervical does not enter the nerve in only 12 cases out of 166.

THE RADIAL NERVE

The radial nerve (musculo-spiral) is usually described as one of the two terminal branches of the posterior fasciculus of the brachial plexus produced by the division of the fasciculus into radial and axillary.

In my series the radial nerve is formed by the division of the posterior fasciculus of the plexus in 138 plexuses, or 79.76 per cent of the 173 satisfactory records for this nerve (figs. 1 to 5).

There are, as already noted in connection with the dorsal fasciculus, 36 plexuses in which there is no true dorsal trunk but the radial and axillary nerves are formed by the union of dorsal branches of the plexus. There are 35 of these cases where the record for the radial nerve is satisfactory and in these cases the radial nerve is formed by the union of two heads.

In 19 of these a cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks, after giving off the axillary nerve forms the cephalic head of the radial nerve. This joins the caudal head of the radial which is the whole of the dorsal division of the caudal trunk (fig. 6). The dorsal division of the intermediate trunk in two of these and the caudal head of the radial nerve in another sends a branch to the axillary nerve. In one of these the dorsal division of the caudal trunk is represented by two branches, one from the eighth cervical and one from the first thoracic nerve. In another the dorsal division of the caudal trunk comes from the eighth cervical only. More than the usual amount of connective tissue had been removed in this case so that three of the branches for the muscles of the arm that ordinarily arise from the radial came from its heads, two from the caudal head and one from the cephalic head. In two of the above 19 cases the radial nerve receives a branch from the medial fasciculus of the plexus, in one as a separate branch and in the other as a branch of the medial brachial cutaneous nerve that comes from the medial fasciculus. In 4 of the above instances, the radial gives off the thoracodorsal nerve.

In 10 cases the dorsal division of the cephalic trunk, after giving off the axillary nerve forms the cephalic head of the radial nerve. In two of these the dorsal division of the cephalic trunk

is formed by the union of the dorsal divisions of the fifth and sixth cervical nerves (fig. 15). In the above 10 instances the caudal head of the radial nerve is formed by the union of the dorsal divisions of the intermediate and caudal trunks (fig. 7). In one of these the dorsal division of the caudal trunk is from the eighth cervical nerve only, but in this case the radial nerve receives a branch from the medial fasciculus and it also gives off the thoracodorsal nerve.

In another case the cephalic head of the radial nerve is formed as above but the caudal head is formed by the dorsal division of the intermediate trunk being joined by the dorsal division of the eighth cervical nerve and more distad by the dorsal division of the first thoracic nerve.

In 4 cases the cephalic head of the radial nerve is formed by the dorsal division of the cephalic trunk after giving off the axillary nerve joining the dorsal division of the intermediate trunk. The caudal head of the radial nerve in these cases is formed by the dorsal division of the caudal trunk.

In one instance the cephalic head of the radial nerve is formed by the dorsal division of the cephalic trunk joining the dorsal division of the intermediate trunk after this has given off the axillary nerve. The caudal head of the radial is the dorsal division of the caudal trunk.

In 169, or 97.68 per cent of the cases, none of the nerves of the plexus can be excluded from sending fibers to the radial. In 109, the fourth, fifth, sixth, seventh and eighth cervical and first thoracic might contribute and in 60 the fourth cervical did not enter the plexus. In 4 instances, there was no dorsal branch from the first thoracic nerve so that in these this could be positively excluded.

Wichmann reports from the literature that fibers from the fifth, sixth, seventh and eighth unite to form the radial nerve in 98 cases; from the sixth, seventh and eighth in 27 cases; from the fifth, sixth, seventh and eighth cervical and first thoracic in 25 cases; the sixth, seventh and eighth cervical and first thoracic in 3; and the fifth, sixth and seventh; the sixth and seventh; and the seventh and eighth in one case each.

Schumacher found the fifth, sixth, seventh, eighth cervical and first thoracic 7 times and the fifth, sixth, seventh and eighth cervical nerves 3 times out of 10 cases.

In comparing my findings with those given above, I can only refer again to what I said concerning the first thoracic nerve when discussing the posterior fasciculus of the plexus.

THE AXILLARY NERVE

The axillary nerve (circumflex) is usually described as one of the terminal branches of the posterior fasciculus of the brachial plexus.

As noted in connection with the radial nerve, this is the condition found in 138 of the 173 satisfactory records in my series, or in 79.76 per cent. In 67 of these, or 38.72 per cent, the axillary is a single nerve (fig. 2); in 57, or 32.94 per cent, it gives off the axillary subscapular nerve to the teres major and axillary border of the subscapularis muscles (fig. 1); in 6 it gives off both the axillary subscapular nerves and the thoracodorsal nerve (fig. 6); in 4 it gives off the axillary subscapular and another separate branch to the axillary border of the subscapularis muscle (fig. 3); in another the axillary subscapular nerve and two additional branches to the subscapularis muscle; in 2 others both the axillary subscapular and thoracodorsal and a branch to the subscapularis muscle; and in another it gives off a branch to the axillary subscapular nerve.

In 35 of the 36 plexuses where there is no posterior fasciculus, the record for the axillary is satisfactory. In 16 of these it arises from a cord formed by the union of the dorsal branches of the cephalic and intermediate trunks (fig. 6). In 6 of these it is single, in 9 it gives off the axillary subscapular nerve, and in one both the axillary subscapular and the thoracodorsal nerve.

In 16 other cases of this group it arises from the dorsal division of the cephalic trunk (fig. 7). In 3 of these the dorsal division of the cephalic trunk is formed by the union of the dorsal divisions of the fifth and sixth cervical nerves. In 7 of these it is single, and in 8 it gives off the axillary subscapular nerve. In one of these it receives a small branch from the axillary subscapular nerve.

In 2 other cases the axillary nerve arises from the dorsal division of the cephalic trunk and receives a branch. This comes from the trunk formed by the union of the dorsal branches of the intermediate and caudal trunks in one case and from the dorsal division of the intermediate trunk in the other case and gives off the thoracodorsal nerve before joining the axillary.

In the other one of the 35 cases, the axillary nerve arises from the dorsal division of the intermediate trunk.

The axillary nerve gives off no other nerves in 80 cases, or 46.24 per cent. The axillary subscapular nerve arises from it in 89 or 55.44 per cent.

It will be noted that in nearly 80 per cent of the above cases the nerve arises from the dorsal fasciculus of the plexus and in these cases it is not possible to tell whether or not all of the cervical nerves that go to the dorsal fasciculus send branches to the axillary nerve. From my maceration experiments I do not believe that as a rule they do. At the same time I cannot now exclude any.

The axillary nerve may receive fibers from any of the nerves from the fourth cervical to the first thoracic inclusive, in 92 instances, 53.17 per cent; from the fifth cervical to the first thoracic in 44 cases, 25.43 per cent; from the fourth to the eighth cervical in one case; from the fifth to the eighth cervical in 2 cases; from the fourth to the seventh cervical in 10; from the fifth to the seventh cervical in 6; from the fourth to the sixth cervical in 6; and from the fifth and sixth cervical nerves in 9 cases.

Wichmann has collected from the literature 142 cases. In 112 of these the fifth and sixth cervical nerves only entered the plexus; in 21 the fifth, sixth and seventh; in 7 the fifth, sixth, seventh and eighth cervical and first thoracic; and in 6 cases the fifth only. Schumacher in his 10 cases found the fifth and sixth cervical 7 times and the fifth, sixth and seventh 3 times. From my experience so far with macerated plexuses I am inclined to believe that the eighth cervical and first thoracic will be found sending fibers to the nerve seldom if at all. In what proportion the seventh will be found, I am unable to say from my maceration experiments. It appears to be present somewhat more often than previously reported.

THE SUBSCAPULAR GROUP OF NERVES

In this group are included the nerves to the subscapularis, the teres major and the latissimus dorsi muscles. There has been some confusion in regard to the names. The nerve or nerves that supply the cephalic and middle parts of the subscapularis muscle was formerly designated as the upper subscapular nerve or nerves. To this group I shall apply the name subscapular nerve, 1, 2 or 3, according to the number found. The nerve that supplies the teres major muscle and the axillary border of the subscapularis muscle and that was formerly called the lower subscapular nerve I shall call the axillary subscapular nerve. The nerve that supplies the latissimus dorsi muscle and that was formerly known as the middle or long subscapular nerve, I shall follow the B. N. A. in calling the thoracodorsal nerve. In some cases the branch or branches that the axillary subscapular usually supplies to the axillary border of the subscapularis muscle are given off separately. I have designated these as the subscapular branches of the axillary subscapular nerve.

THE SUBSCAPULAR NERVES

The subscapular nerve (upper subscapular) is usually described as arising from the dorsal division of the cephalic trunk and as being often double.

In my series there are 157 satisfactory records of this nerve. In 84, or 53.50 per cent of them, there is a single nerve (fig. 4), in 64, or 40.76 per cent, there are two nerves and in 9, or 5.73 per cent, there are 3 nerves.

In 40 of the plexuses in which the subscapular is represented by a single branch, this arises from the dorsal division of the cephalic trunk of the plexus (fig. 8). In 2 of these there is another nerve to the subscapularis muscle arising from the axillary nerve, which both from its origin and relations I have considered as one of the branches that the axillary subscapular nerve sends to the subscapular muscle and which in this case arises separately. One of the 40 single nerves arises by two roots and another comes from the suprascapular nerve.

In 17 plexuses where the nerve is single it arises from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks (fig. 11).

In 13 instances the single nerve arises from the posterior fasciculus of the plexus (fig. 21). In 2 of these there is an additional branch that I regard as belonging to the axillary subscapular nerve.

In 7 cases the nerve arises from the dorsal division of the intermediate trunk (seventh cervical nerve) and in one case from the ventral division of this.

In one plexus the subscapular nerve comes from the fifth cervical nerve; in one by two roots, one from the cephalic trunk and the other from the dorsal division of this; in another, by three roots, one from the cephalic trunk with the suprascapular nerve, one from the lateral fasciculus of the plexus, and one from the intermediate trunk. In one plexus, the nerve comes from the dorsal division of the cord formed by the union of the cephalic and intermediate trunks and in one it comes from a cord formed by the union of the intermediate and caudal trunks. In one instance the nerve arises from the radial nerve. In this case the axillary subscapular and the thoracodorsal nerves also arise from the radial nerve.

In 7 of the plexuses where there is a single nerve this divides almost immediately after its origin into 2 branches in 5 cases and 3 branches in 2. The nerve frequently breaks up into several branches before entering the muscle but my records do not show the method of branching.

In 21 of the 64 instances where there are two subscapular nerves both arise from the dorsal division of the cephalic trunk (fig. 2). In two of these there is another separate nerve to the subscapularis muscle that comes from the axillary nerve and which I have considered as a part of the axillary subscapular nerve.

In 13 cases, one of the two subscapular nerves comes from the dorsal division of the cephalic trunk and the second from the posterior fasciculus of the plexus (fig. 1).

In 7 cases both branches come from the cord formed by the

union of the dorsal divisions of the cephalic and intermediate trunks.

In 6 plexuses one branch comes from the cord just mentioned and the second from the dorsal division of the cephalic trunk. In one of these one of the branches from the dorsal division of the cephalic trunk arises with or from the suprascapular nerve.

In 9 instances both nerves come from the posterior fasciculus (fig. 20). In one of these there is another subscapular nerve coming from the axillary but classified as a part of the axillary subscapular nerve.

In 4 cases one nerve comes from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks and the other from the posterior fasciculus (fig. 19).

There are 2 cases in which one branch comes from the fifth cervical nerve and the other from the dorsal division of the cephalic trunk. This second branch sends a small filament to join a cord from which the axillary subscapular and the thoracodorsal nerves arise. In one case one branch comes from the ventral and one from the dorsal division of the cephalic trunk and in one instance one comes from the cephalic trunk and one from the posterior fasciculus.

There are 9 plexuses in which there are three branches. In 5 of these two of the branches arise from the dorsal division of the cephalic trunk. The third branch in one of these comes from the dorsal division of the fifth cervical nerve; in one it comes from the dorsal division of the cephalic trunk like the other two branches but has a second root from the dorsal division of the intermediate trunk in common with the thoracodorsal nerve (fig. 10); in one, the third branch comes from the trunk formed by the union of the dorsal divisions of the cephalic and intermediate trunks; in another from the posterior fasciculus; and in the last of this group from the axillary nerve. In one plexus one branch arises from the dorsal division of the intermediate trunk and two come from the posterior fasciculus of the plexus. In one case one branch comes from the cord formed by the union of the cephalic and intermediate trunks, one from the dorsal division of this, and a third from the posterior fasciculus. In one

plexus all three branches come from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks and in another one branch from this same cord and the other two from the posterior fasciculus of the plexus. In the first of these two last mentioned cases, there are two and in the second case there is one additional nerve to the subscapularis muscle but these are grouped with the axillary subscapular nerve.

From the above it will be seen that one subscapular nerve arises from the dorsal division of the cephalic trunk in 89 cases, or 56.68 per cent of the cases; from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks in 30 or in 19.10 per cent; from the posterior fasciculus in 44 or in 28.02 per cent; and from various other divisions of the plexus in the other cases.

The subscapular nerve arises from the fifth cervical nerve in one case and from the seventh in 8 instances. In all the other cases it arises in such a way that two or more nerves may contribute fibers to it. In 40 cases the fourth, fifth and sixth may contribute and in 28 the fifth and sixth. In 22 instances the fourth fifth, sixth and seventh may send fibers and in 15 the fifth, sixth and seventh. In 34 plexuses all of the nerves from the fourth cervical to the first thoracic and in 9 from the fifth cervical to the first thoracic may contribute. Herringham ('87) reports on 41 cases and states that in the majority of cases the fifth nerve only sends fibers to the subscapular, but that often the sixth contributes but in no instance the seventh and eighth cervical. I found 8 plexuses in which the seventh cervical was the only nerve that could send fibers to it.

THE AXILLARY SUBSCAPULAR NERVE

The axillary subscapular nerve (lower subscapular) is usually described as arising from the posterior fasciculus of the plexus or one of the roots of this. It supplies the teres major muscle and the axillary border of the subscapularis muscle.

Among the 157 satisfactory records of the axillary subscapular nerves in my series, the nerve arises directly from the posterior fasciculus in 48 cases (fig. 2). In one of these it receives a branch

from the subscapular nerve and gives off a small branch to the latissimus dorsi muscle. In 5, it is formed from a common trunk with the thoracodorsal nerve. In 68 other cases it arises from the axillary nerve which takes origin from the posterior fasciculus (fig. 1), and in one from the radial nerve that arises from the posterior fasciculus. Its origin is directly or indirectly from the posterior fasciculus in 117 cases, or in 74.52 per cent of the cases.

It arises directly from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks in 12 cases (fig. 13), and from the axillary nerve that takes origin from this cord in 9 others (fig. 6), making a total of 21 or in 13.37 per cent of the 157 cases.

It comes from the dorsal division of the cephalic trunk in 3 cases directly (fig. 10), and in 7 others from the axillary nerve that takes origin from the dorsal division of the cephalic trunk (fig. 23), making 10 in all.

In one other case it arises from the axillary nerve and this nerve arises by two roots, one from the dorsal division of the cephalic trunk and one from the dorsal division of the intermediate trunk.

In one plexus it arises from the dorsal division of the intermediate trunk and in 2 from the cord formed by the union of the dorsal divisions of the caudal and intermediate trunks.

In 5 instances it arises by two heads. In 4 of these one comes from the dorsal division of the cephalic, and one from the dorsal division of the intermediate trunk (fig. 7). In one of these the thoracodorsal nerve arises in common with the axillary subscapular nerve. In the fifth case, one root comes from the cord formed by the union of the dorsal branches of the cephalic and intermediate trunks and the other from the dorsal division of the caudal trunk. Immediately after its origin, the nerve receives a branch from the thoracodorsal nerve.

It will be noted then that the axillary subscapular nerve arises directly from one of the dorsal divisions of the brachial plexus in 71 instances or 45.22 per cent, and from the axillary nerve in 85 instances or 54.14 per cent.

From those cases in which my records show the branches of the axillary subscapular nerve to the subscapularis muscle, it will be seen that in 21 instances there is a single branch; in 15 there are 2 branches, besides 3 cases in which there is one branch from the axillary subscapular nerve and a second branch from the axillary nerve close to it, and one case in which there are two branches from the axillary nerve to the subscapularis muscle, and none from the axillary subscapular nerve.

There are 5 cases in which there are three nerves to the axillary part of the subscapularis muscle. In 2 of these all three came from the axillary subscapular, in 2 cases two come from the axillary subscapular, and the third from the posterior fasciculus, and in the fifth case two branches come from the axillary subscapular and the third from the axillary nerve close to it.

In one plexus there are four branches from the axillary subscapular nerve to the axillary part of the subscapularis muscle.

There are 115 plexuses in which I cannot be sure that fibers from all the nerves of the plexus may not enter the axillary subscapular nerve. In 79 of these the nerves from the fourth cervical to the first thoracic may send fibers and in 36 from the fifth cervical to the first thoracic. In one case fibers may come from the fourth to the eighth cervical and in two from the fifth to the eighth; in 14 from the fourth to the seventh cervical and in 12 from the fifth to the seventh. In 6 cases the fourth, fifth and sixth cervical are the only nerves and in 4 the fifth and sixth. In one the seventh cervical and in 2 the seventh and eighth cervical and first thoracic may contribute.

Herringham was able to exclude the eighth cervical and first thoracic nerves from the axillary subscapular in all of his 41 cases. He found it arising from the fifth, sixth and seventh cervical nerves in 3 cases; the sixth and seventh in 9. The seventh nerve contributing to the plexus only in these 12 cases. The nerve arises from the fifth and sixth cervical nerves in 9 cases and from a trunk formed by branches of these nerves in 13 cases or 22 in all where the fifth and sixth may have formed the nerve. The sixth alone in 4 and the fifth alone in 3 cases formed this nerve.

THE THORACODORSAL NERVE

The thoracodorsal (middle or long subscapular) nerve is usually described as arising from the posterior fasciculus of the plexus.

In my series of 161 satisfactory records for this nerve it arises singly from the posterior fasciculus in 93 instances or 57.76 per cent (fig. 2). In 2 of these the posterior fasciculus receives no fibers from the first thoracic nerve. In 4 of the above, in addition to its origin from the posterior fasciculus, the nerve has a second root, coming from the dorsal division of the caudal trunk in 2, from the dorsal division of the intermediate trunk in one, and from the posterior fasciculus itself in one.

In 7 cases the nerve arises from the posterior fasciculus by a stem common to it and the axillary subscapular nerve. In 9 plexuses the nerve arises from the radial nerve which in 5 of these is one of the terminal divisions of the posterior fasciculus and in 3 others the radial nerve is formed by a branch from the trunk produced by the union of the dorsal divisions of the cephalic and intermediate trunks after giving off the axillary nerve joining the dorsal division of the caudal trunk. In another case the radial nerve from which the thoracodorsal arises is formed by a branch from the dorsal division of the cephalic trunk, after giving off the axillary nerve, joining the cord formed by the union of the dorsal division of the intermediate trunk and a dorsal branch of the eighth cervical and joined by a branch from the caudal trunk (fig. 28).

In 9 plexuses the nerve arises from the axillary nerve. In 8 of these this is one of the terminal divisions of the posterior fasciculus (fig. 17). In the other case the axillary nerve comes from the trunk formed by the union of the dorsal branches of the cephalic and intermediate trunks but the thoracodorsal nerve receives almost immediately after its origin from the axillary a branch from the dorsal division of the caudal trunk.

The thoracodorsal nerve arises either directly or indirectly from the posterior fasciculus of the plexus in 113 plexuses, or in 70.19 per cent.

In 18 instances the thoracodorsal nerve arises from the cord formed by the union of the dorsal divisions of the cephalic and

intermediate trunks. In 3 of these it comes from a stem common to it and the axillary subscapular nerve. In one other case it gives off a small branch to supply the teres major muscle which is also supplied by the axillary subscapular.

In 10 plexuses the nerve comes from the dorsal division of the intermediate trunk, that is the seventh cervical nerve (fig. 15). In one of these it does not arise directly but comes from a branch that forms one of the heads of the axillary nerve.

The nerve arises in 4 cases by two roots, one from the dorsal division of the cephalic trunk and one from the dorsal division of the intermediate trunk (fig. 18). In one of these the stem formed by the union of the two roots gives origin also to the subscapular nerve and in another case to the axillary subscapular.

In 3 cases it arises from the dorsal division of the cephalic trunk. One of these gives off a branch that joins the axillary subscapular nerve.

The thoracodorsal nerve arises from the cord formed by the union of the dorsal divisions of the intermediate and caudal trunks in 6 instances (fig. 7), and in another it arises by two roots, one from the dorsal division of the intermediate trunk and one from the dorsal division of the caudal trunk. In one case the nerve arises from the dorsal division of the caudal trunk.

In 115 cases, or 71.42 per cent of this series, no nerve that enters the plexus can be excluded from sending fibers to the thoracodorsal nerve. In 73, the spinal nerves from the fourth cervical to the first thoracic inclusive enter the plexus and in 35 from the fifth cervical to the first thoracic. In one case all of the nerves from the fourth cervical to the eighth cervical and in 2 others from the fifth to the eighth.

In 14 cases the nerves from the fourth to the seventh cervical may contribute, in 8 from the fifth cervical to the seventh. In 3 instances the fifth and sixth cervical nerves; in 10 the seventh cervical; in 7 the seventh and eighth cervical and first thoracic; in one the eighth cervical and first thoracic are the nerves that may send fibers to the thoracodorsal nerve.

Herringham, from his study of 42 plexuses, found that the seventh cervical alone sent fibers to the thoracodorsal nerve in

21 cases; the seventh and eighth in 13; sixth, seventh and eighth in one; the sixth, and seventh in 3; the fifth, sixth and seventh in 3 and the fifth, and sixth in one case. It will be noted that from only one case in his series was the seventh lacking. In my series there are 4 cases in which the seventh cervical nerve does not enter the thoracodorsal nerve. In 3 of these it comes from the fifth and sixth cervical nerve as in Herringham's case and in the other case from the eighth cervical and first thoracic nerves.

SUMMARY

1. The 175 brachial plexuses studied can be divided into three groups according to the nerves joining the cephalic border of the plexus.

2. Those plexuses that receive a branch from the fourth cervical nerve are grouped together as group 1. Over 62 per cent of the plexuses are formed in this way (fig. 1).

3. Those plexuses that receive no branch from the fourth cervical nerve but in which the whole of the ventral division of the fifth cervical nerve joins the plexus form a second group. Nearly 30 per cent of those studied are in this group (fig. 2).

4. Plexuses that receive no branch from the fourth cervical and in which a part of the fifth cervical nerve helps to form the cervical plexus form a third group that contains a little over 7 per cent of the plexuses (fig. 3).

5. The plexuses studied do not show that sex, color or side of the body have much if any influence upon the classification into these groups.

6. Plexuses that receive a branch from the fourth cervical nerve are more cephalic as regards the spinal column and those in which only part of the fifth enters the plexus are more caudal. The larger the branch from the fourth nerve to the brachial plexus the more cephalic the plexus and conversely, the larger the branch from the fifth to the cervical plexus, the more caudal the plexus.

7. The relative size of the nerves, especially those in the center of the plexus, is found to have but little value in estimating the position of the plexus as cephalic or caudal. This is because

the diameter is dependent upon so many other factors than the nerve fibers.

8. Maceration shows how very complexly the nerve bundles interlace and join and how difficult it is, even when the epineurium is removed by maceration, to trace the bundles.

9. By anatomical methods the fibers of a given spinal nerve can rarely be traced through the plexus to their ultimate distribution. In only a few of the branches of the plexus can it be determined anatomically which spinal nerves send fibers to them.

10. The nerves cephalic to the seventh cervical join to form a cephalic trunk which then divides into dorsal and ventral branches in nearly 90 per cent of the cases.

11. The nerves caudal to the seventh unite to form a caudal trunk in over 95 per cent of the plexuses.

12. The seventh cervical remains single and forms the intermediate trunk in all cases.

13. In over 80 per cent of the cases the lateral fasciculus is formed by the union of the ventral branches of the cephalic and intermediate trunks.

14. The medial fasciculus is composed of the ventral branch of the caudal trunk in over 95 per cent of the plexuses.

15. In nearly 70 per cent of the plexuses the posterior fasciculus is formed by the union of the dorsal divisions of the cephalic, intermediate and caudal trunks. In over 20 per cent of the plexuses there is no true posterior fasciculus formed.

16. The plexuses are subdivided into seven subgroups or types based upon the nerves entering the medial or the lateral fasciculus.

17. In type A, group 1, the fourth cervical nerve sends a branch to the plexus and the seventh cervical nerve gives a branch to the medial fasciculus. There are less than 3 per cent of the plexuses of this type (fig. 4).

18. In type B, group 1, the fourth cervical nerve sends a branch to the plexus and the medial fasciculus receives no branch from the seventh cervical nerve. Over 57 per cent of the plexuses are of this type (fig. 1).

19. Type C, group 1, differs from the preceding in having a branch from the caudal trunk or eighth cervical nerve to the

lateral fasciculus or seventh cervical nerve. There are only a little over 2 per cent of the plexuses of this type (fig. 6).

20. In type D, group 2, like type A, there is a branch from the intermediate trunk to the medial fasciculus but there is no branch from the fourth cervical in this type. There is but one example of this arrangement or slightly over one half of one per cent (fig. 7).

21. Type E, group 2, is exactly like type B, group 1, except there is no branch from the fourth cervical nerve to the plexus. Nearly 30 per cent of the plexuses are of this type (fig. 2).

22. Type F, group 3, differs from the preceding only in that but part of the fifth cervical joins the plexus. There is a branch from the fifth cervical nerve to the cervical plexus. Slightly less than 7 per cent of the plexuses are arranged in this way (fig. 3).

23. In type G, group 3, there is a branch from the caudal trunk to the lateral fasciculus, otherwise this is just like the preceding. There is only one specimen of this type (fig. 8).

24. The typical plexuses are those of type B (fig. 1), type E (fig. 2) and type F (fig. 3).

25. In the 63 bodies, 126 plexuses where there are complete records from both sides of the body, the same type of plexus is found on both sides in over 61 per cent.

26. Symmetry is more common than asymmetry in the ratio of about 3 to 2.

27. Asymmetry is found very slightly more often in females than in males.

28. Asymmetry is found most often in white males, least often in colored males, and more often among colored than white females.

29. Symmetry occurred only in the plexuses of type B, E and F which are the most common and typical plexuses of the three groups.

30. Slightly over 71 per cent of the type B plexuses are symmetrical, 60 per cent of the type F and about 54 per cent of the type E.

31. Where asymmetry occurs, the cephalic type of plexus is found on the right side in over 62 per cent.

32. In over 54 per cent of the cases the cephalic group, type B on one side is associated with an intermediate group, type E on the other.

33. In 12.50 per cent the cephalic group B is on one side and the caudal group F on the other.

34. The ulnar nerve is formed by the division of the medial fasciculus of the plexus into medial head of the median nerve and the ulnar nerve in over 97 per cent of the cases.

35. There is a lateral head to the ulnar nerve arising from the lateral fasciculus of the plexus, from the lateral head of the median nerve or from the seventh cervical nerve in about 60 per cent of the cases.

36. The ulnar nerve receives its fibers from the eighth cervical and first thoracic nerves probably in all cases, and in those with a lateral head, some fibers from the seventh cervical and possibly some other nerves of the plexus send fibers to it.

37. In the cephalic group of plexuses the ulnar nerve appears to receive fibers from spinal nerves cephalic to the eighth cervical nerve more frequently than in the caudal group.

38. In over 86 per cent of the cases the median nerve is formed by the union of two heads, a lateral head from the lateral fasciculus of the plexus, a medial head from the medial fasciculus.

39. In only one of my cases can I be sure that any nerve that contributes to the brachial plexus did not also send fibers to the median nerve.

40. The musculocutaneous nerve arises from the division of the lateral fasciculus of the plexus into medial head of the median and musculocutaneous nerve in over 88 per cent of the plexuses.

41. In the arm the musculocutaneous gives off a branch that joins the median nerve in 24 per cent of the cases. This is probably a greater percentage than would be found if complete records through the arm had been available for all the cases.

42. The musculocutaneous nerve may receive its fibers from the fourth, fifth, sixth and seventh cervical nerves in 56 per cent of the cases and from the fifth, sixth and seventh cervical nerves only in over 30 per cent, that is fifth, sixth and seventh cervical nerves may contribute in over 86 per cent.

43. The nerve to the coracobrachialis muscle arises from the musculocutaneous in over 40 per cent of the plexuses and from the lateral fasciculus of the plexus in over 20 per cent.

44. The nerve to the coracobrachialis muscle is represented by a single branch in over 55 per cent of the cases; by 2 branches in over 31 per cent; by three branches in over 10 per cent; and by four branches in over 2.5 per cent.

45. In over 84 per cent none of the nerves of the plexus cephalic to the eighth cervical can be excluded from sending fibers to the coracobrachialis muscle.

46. The suprascapular nerve arises from the cephalic trunk of the plexus in over 62 per cent of the cases and from the cephalic trunk or its dorsal or ventral division in over 82 per cent. Fibers of the fifth and sixth cervical nerves may enter the nerve in all of these cases and the fourth also may send fibers in two-thirds of them.

47. The subclavius nerve in nearly 50 per cent of the cases arises from the cephalic trunk of the plexus or its ventral branch. In nearly two-thirds of these the fourth, fifth and sixth cervical nerves may send fibers to it. In over 26 per cent of the cases, it arises from the fifth cervical nerve only and in over 21 per cent it may receive fibers from both fourth and fifth cervical nerves.

48. The subclavius nerve arises as a single branch in over 65 per cent of the cases and from a stem that communicates with the phrenic nerve in over 28 per cent of the cases.

49. The medial brachial cutaneous nerve is represented by a single branch in over 82 per cent of the cases.

50. In over 73 per cent of the cases, the medial brachial cutaneous nerve arises separately. When combined it is most often with the medial antibrachial cutaneous nerve.

51. The fibers of both the first thoracic and eighth cervical nerves may enter the medial brachial cutaneous nerve in over 95 per cent of the cases.

52. Anastomoses of the medial brachial cutaneous nerve with the intercostobrachial is found in over 60 per cent of the cases.

53. In over 82 per cent of the plexuses the medial antibrachial

cutaneous nerve arises from the medial fasciculus of the plexus and in over 10 per cent from the caudal trunk.

54. In over 97 per cent of the cases both the eighth cervical and first thoracic nerves may send fibers to the medial antibrachial cutaneous nerve.

55. The medial anterior thoracic nerve arises from the medial fasciculus in over 69 per cent of the plexuses and from the caudal trunk in over 24 per cent.

56. Both the first thoracic and the eighth cervical may send fibers to the medial anterior thoracic in over 95 per cent of the cases.

57. The lateral anterior thoracic nerve arises from two roots in over 54 per cent of the plexuses, by one root in over 23 per cent and by three roots in nearly 20 per cent.

58. In over 43 per cent of the cases where there is a single root for the lateral anterior thoracic nerve, this comes from the lateral fasciculus of the plexus. In over 78 per cent of the cases where there are two roots, one of them comes from the ventral division of the cephalic trunk and the other from the ventral division of the intermediate trunk (seventh cervical nerve) before these join to form the lateral fasciculus. In over 54 per cent of the cases where there are three roots one arises from the ventral division of the cephalic trunk and the other two from the ventral division of the intermediate trunk of the plexus.

59. In over 52 per cent of the cases the fourth to the seventh cervical nerves can none of them be excluded from the lateral anterior thoracic nerve and in over 30 per cent, the fifth to the seventh. All of the nerves cephalic to the eighth cervical nerve may send fibers to the lateral anterior thoracic nerve in over 83 per cent of the cases.

60. The radial nerve arises as one of the terminal divisions of the plexus in over 79 per cent of the cases. In the other cases the radial nerve is formed by the union of two heads.

61. In 63 per cent the fourth cervical to the first thoracic nerves can none of them be excluded from the radial nerve and in over 34 per cent the fourth cervical to the first thoracic can not be excluded, that is, in 97 per cent of the plexuses none of the nerves that entered the plexus can be excluded.

62. The axillary nerve arises as one of the terminal divisions of the plexus in nearly 80 per cent of the cases. In over 9 per cent it arises from the cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks and in the same number of cases from the dorsal division of the cephalic trunk.

63. In over 46 per cent of the cases the axillary nerve gives off none of the other nerves of the plexus but in over 55 per cent it gives origin to the axillary subscapular nerve.

64. None of the nerves from the fourth cervical to the first thoracic can be excluded from the axillary nerve in over 53 per cent of the cases and from the fifth cervical to the first thoracic in over 25 per cent. None of the nerves that enter the plexus can be excluded in over 78 per cent of the plexuses.

65. The subscapular nerve occurs as a single nerve in over 53 per cent of the plexuses. There are two nerves in over 40 per cent and three nerves in over 5 per cent of the plexuses.

66. One of the subscapular nerves arises from the dorsal division of the cephalic trunk of the plexus in over 56 per cent of the cases; from the dorsal division of the intermediate trunk in over 19 per cent and from the posterior fasciculus in over 28 per cent.

67. In over 61 per cent of the cases the fourth cervical nerve may send fibers to the subscapular nerve; in over 94 per cent the fifth and sixth cervical nerves; and in over 50 per cent the seventh cervical nerve.

68. The axillary subscapular nerve arises directly from the posterior fasciculus of the plexus or from the axillary nerve or the radial nerve that arise from the posterior fasciculus in over 74 per cent of the cases. It comes from the dorsocephalic cord formed by the union of the dorsal divisions of the cephalic and intermediate trunks in over 13 per cent.

69. The axillary subscapular nerve arises directly from one of the dorsal divisions of the brachial plexus in over 45 per cent of the cases and from the axillary nerve in over 54 per cent.

70. None of the nerves of the plexus can be positively excluded from the axillary subscapular in over 72 per cent of the cases.

The nerves caudal to the seventh cervical can be excluded in 23 per cent of the cases.

71. The thoracodorsal nerve arises either directly from the posterior fasciculus of the plexus or from a nerve that takes origin from the posterior fasciculus in over 70 per cent of the plexuses. In over 5.5 per cent of the cases it arises from the radial nerve and in the same number from the axillary nerve.

72. In over 71 per cent, none of the nerves of the plexus can be positively excluded from sending fibers to the thoracodorsal nerve.

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TABLE 1

Showing the distribution of 175 brachial plexuses as to sex, color and side of the body

	MALE		FEMALE		MALE AND FEMALE	
	Number	Per cent	Number	Per cent	Number	Per cent
White	65	37.14	20	11.42	85	48.57
Colored	49	28.00	41	23.42	90	51.42
Right	56	32.00	31	17.71	87	49.71
Left	58	33.14	30	17.14	88	50.28
White, Right	33	18.85	9	5.14	42	24.00
White, Left	32	18.28	11	6.28	43	24.57
Colored, Right	23	13.14	22	12.57	45	25.71
Colored, Left	26	14.85	19	10.85	45	25.71
Total	114	65.14	61	34.85	175	100.00

TABLE 2

Showing the distribution as to sex, color, and side of the body of the 110 brachial plexuses of Group 1

	MALE		FEMALE		MALE AND FEMALE	
	Number	Per cent	Number	Per cent	Number	Per cent
White	41	37.27	15	13.63	56	50.90
Colored	29	26.36	25	22.72	54	49.09
Right	34	30.90	20	18.18	54	49.09
Left	36	32.72	20	18.18	56	50.90
White, Right	20	18.18	6	5.45	26	23.63
White, Left	21	19.09	9	8.18	30	27.27
Colored, Right	14	12.72	14	12.72	28	25.45
Colored, Left	15	13.63	11	10.00	26	23.63
Total	70	63.63	40	36.36	110	100.00

TABLE 3

Showing the distribution as to sex, color, and side of the body of the 52 brachial plexuses of Group 2

	MALE		FEMALE		MALE AND FEMALE	
	Number	Per cent	Number	Per cent	Number	Per cent
White	18	34.61	3	5.76	21	40.38
Colored	17	32.69	14	26.92	31	59.61
Right	19	36.53	9	17.30	28	53.84
Left	16	30.77	8	15.38	24	46.15
White, Right	11	21.15	2	3.84	13	25.00
White, Left	7	13.46	1	1.92	8	15.38
Colored, Right	8	15.38	7	13.46	15	28.82
Colored, Left	9	17.30	7	13.46	16	30.77
Total	35	67.30	17	32.69	52	100.00

TABLE 4

Showing the distribution as to sex, color, and side of the body of the 13 brachial plexuses of Group 3

	MALE		FEMALE		MALE AND FEMALE	
	Number	Per cent	Number	Per cent	Number	Per cent
White	6	46.14	2	15.38	8	61.53
Colored	3	23.07	2	15.38	5	38.46
Right	3	23.07	2	15.38	5	38.46
Left	6	46.14	2	15 38	8	61.53
White, Right	2	15.38	1	7.69	3	23.07
White, Left	4	30.76	1	7.69	5	38.46
Colored, Right	1	7.69	1	7.69	2	15.38
Colored, Left	2	15.38	1	7.69	3	23.07
Total	9	69.23	4	30.76	13	100.00

TABLE 5

Showing distribution among the three groups of the 114 plexuses from male subjects

	GROUP 1		GROUP 2		GROUP 3	
White	41	35.96	18	15.78	6	5.26
Colored	29	25.43	17	14.91	3	2.63
Right	34	29.82	19	16.66	3	2.63
Left	36	31.57	16	14.02	6	5.26
White { Right	20	17.54	11	9.64	2	1 75
White { Left	21	18.42	7	6.14	1	0.87
Colored { Right	14	12.28	8	7.01	4	3.50
Colored { Left	15	13.15	9	7.89	2	1.75
Total	70	61.40	35	30.70	9	7.89

TABLE 6

Showing distribution among the three groups of the 61 plexuses from female subjects

	GROUP 1		GROUP 2		GROUP 3	
White	15	24.59	3	4.92	2	3.27
Colored	25	40.98	14	22.95	2	3.27
Right	20	32.78	9	14.75	2	3.27
Left	20	32.78	8	13.11	2	3.27
White { Right	6	9.83	2	3.27	1	1.63
White { Left	9	14.75	1	1.63	1	1.63
Colored { Right	14	22.95	7	11.47	1	1.63
Colored { Left	11	18.03	7	11.47	1	1.63
Total	40	65.57	17	27.86	4	6.55

TABLE 7

Showing the distribution among the three groups of the 85 plexuses from white subjects

	GROUP 1		GROUP 2		GROUP 3	
Male	41	48.23	18	21.15	6	7.05
Female	15	17.64	3	3.52	2	2.35
Right	26	30.58	13	15.29	3	3.52
Left	30	35.29	8	9.41	5	5.88
Right { Male	20	23.52	11	12.94	2	2.35
Right { Female	6	7.05	2	2.35	1	1.17
Left { Male	21	24.70	7	8.23	4	4.70
Left { Female	9	10.58	1	1.17	1	1.17
Total	56	65.88	21	24.70	8	9.41

TABLE 8

Showing the distribution among the three groups of the 90 plexuses from colored subjects

	GROUP 1		GROUP 2		GROUP 3	
Male	29	32.22	17	18.88	3	3.33
Female	25	27.77	14	15.55	2	2.22
Right	28	31.11	15	16.66	2	2.22
Left	26	28.88	16	17.77	3	3.33
Right { Male	14	15.55	8	8.88	1	1.11
Right { Female	14	15.55	7	7.77	1	1.11
Left { Male	15	16.56	9	10.00	2	2.22
Left { Female	11	12.22	7	7.77	1	1.11
Total	54	59.99	31	34.44	5	5.55

TABLE 9

Showing the distribution among the three groups of the 87 plexuses from the right side of the body

	GROUP 1		GROUP 2		GROUP 3	
Male	34	39.08	19	21.83	3	3.44
Female	20	22.98	9	10.34	2	2.29
White	26	29.88	13	14.94	3	3.44
Colored	28	32.18	15	17.24	2	2.29
Male { White	20	22.58	11	12.64	2	2.29
Male { Colored	14	16.07	8	9.19	1	1.14
Female { White	6	6.89	2	2.29	1	1.14
Female { Colored	14	16.09	7	8.04	1	1.14
Total	54	62.06	28	32.18	5	5.74

TABLE 10

Showing the distribution among the three groups of the 88 plexuses from the left side of the body

	GROUP 1		GROUP 2		GROUP 3	
Male	36	38.63	16	18.18	6	6 81
Female	20	22.72	8	9.09	2	2.27
White	30	34.09	8	9.09	5	5.68
Colored	26	29.54	16	18.18	3	3.40
Male { White	21	23.86	7	7.95	4	4.54
Male { Colored	15	17.04	9	10.22	2	2.27
Female { White	9	10.22	1	1.17	1	1.13
Female { Colored	11	12.50	7	7.95	1	1.13
Total	56	63.63	24	27.27	8	9 09

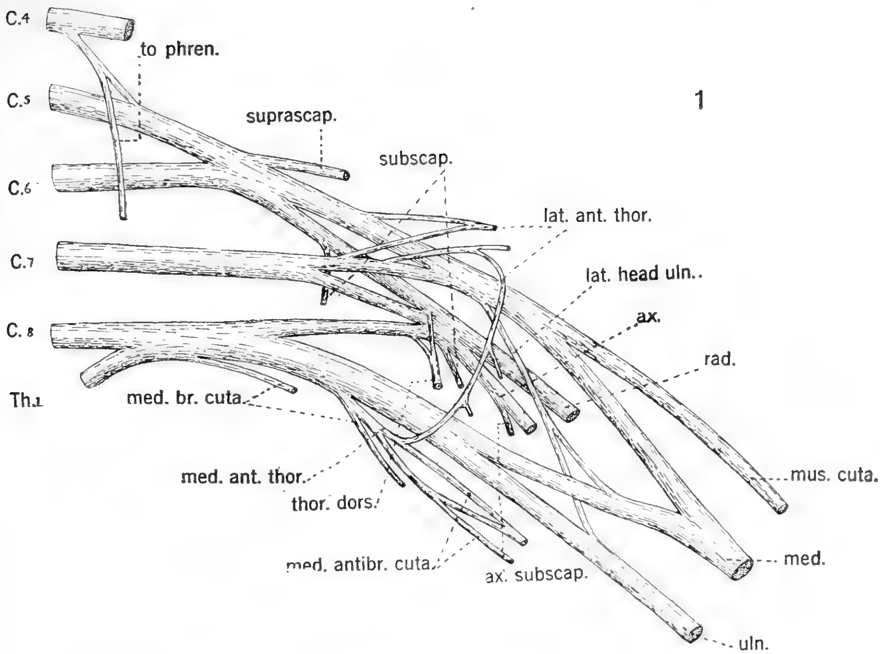
TABLE 11

Showing the relative size of the nerves of the brachial plexus in the 27 cases that were measured

	Type of plexus		Type of plexus
5C < 1T < 6C < 7C = 8C	C	5C = 1T < 6C = 8C < 7C	C
5C < 1T < 6C < 7C = 8C	C	5C = 1T < 6C = 8C < 7C	C
5C < 1T < 6C = 7C < 8C	C	5C = 1T < 6C = 7C = 8C	C
5C < 1T < 6C < 8C < 7C	G	5C = 1T < 6C = 7C = 8C	C
5C < 1T = 6C < 7C = 8C	C	5C = 1T = 6C < 7C = 8C	F
5C < 1T = 6C < 7C = 8C	B	5C = 1T < 7C < 6C < 8C	C
5C < 1T = 6C < 8C < 7C	G	1T < 5C < 6C = 7C < 8C	G
5C < 1T = 6C = 7C = 8C	F	1T < 5C < 6C = 7C < 8C	C
5C < 1T = 7C < 8C < 6C	F	1T < 5C < 8C < 6C = 7C	C
5C < 1T < 8C < 6C < 7C	C	1T < 5C = 8C < 6C < 7C	C
5C < 1T < 8C < 6C = 7C	C	1T < 5C = 8C < 7C < 6C	C
5C = 1T < 6C < 7C < 8C	C	1T < 6C < 5C < 7C = 8C	C
5C = 1T < 6C < 7C < 8C	C	1T < 8C < 5C = 6C = 7C	C
5C = 1T < 6C < 8C < 7C	C		

EXPLANATION OF FIGURES

The drawings for this article, with one exception, were made by Mrs. Kerr. They are semidiagrammatic sketches. The plexuses from the right side have been reversed to render comparison easier. Figures 1 to 8 and 29 are two-thirds natural size and figures 10 to 2 are one-half size. Figure 9 drawn by Miss Whitman from the macerated specimen is reduced to about natural size.



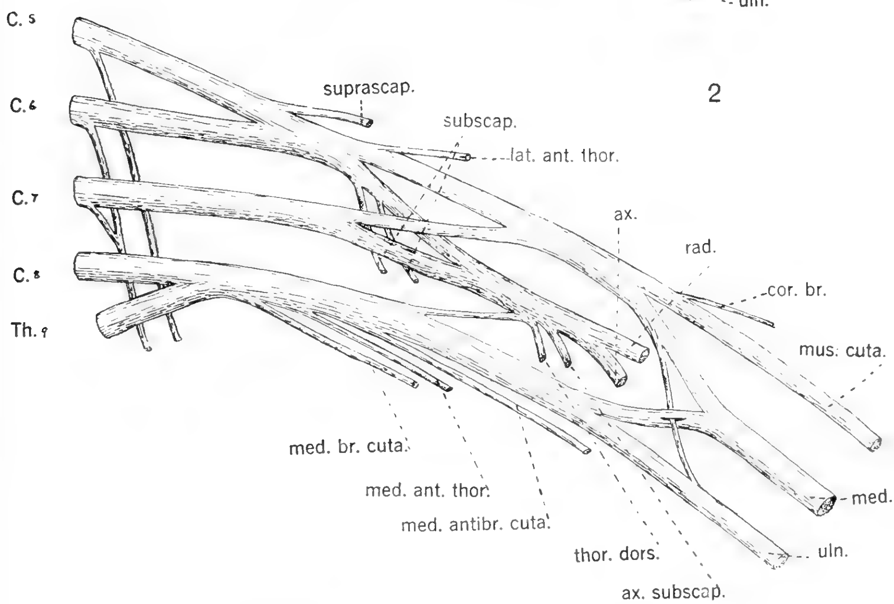
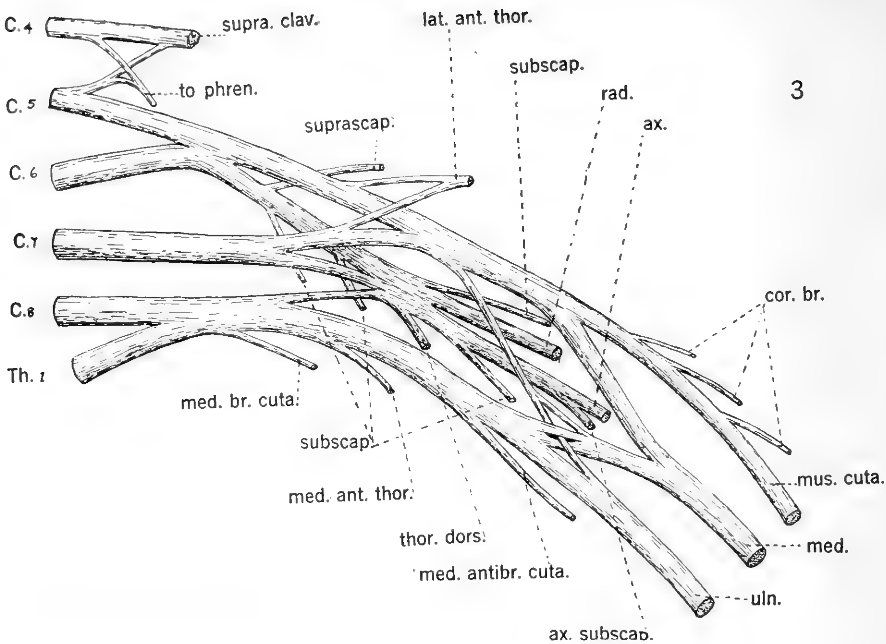


Fig. 1 From the right side of a white female, age 73 years, reversed. Group 1, Type B.

Fig. 2 From the right side of a colored female, age about 60 years, reversed. Group 2, Type E.



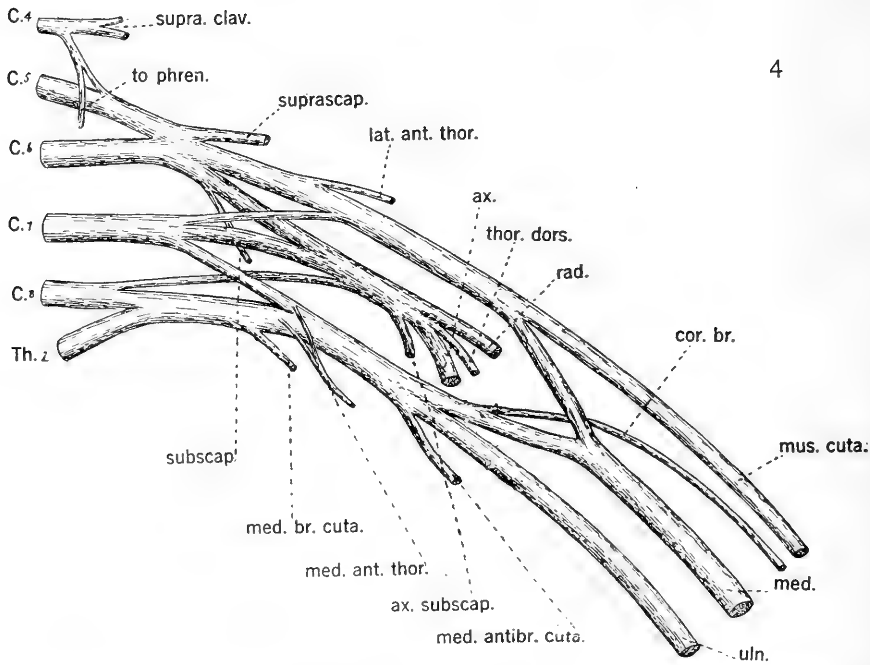
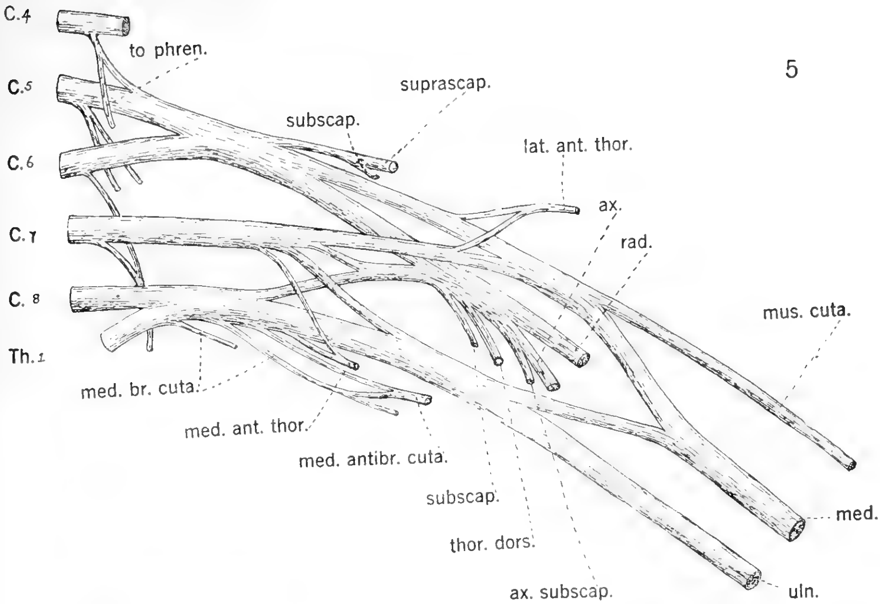


Fig. 3 From the right side of a white male, age about 35 years, reversed. Group 3, Type F.

Fig. 4 From the right side of a white female, age 95 years, reversed. Group 1, Type A.



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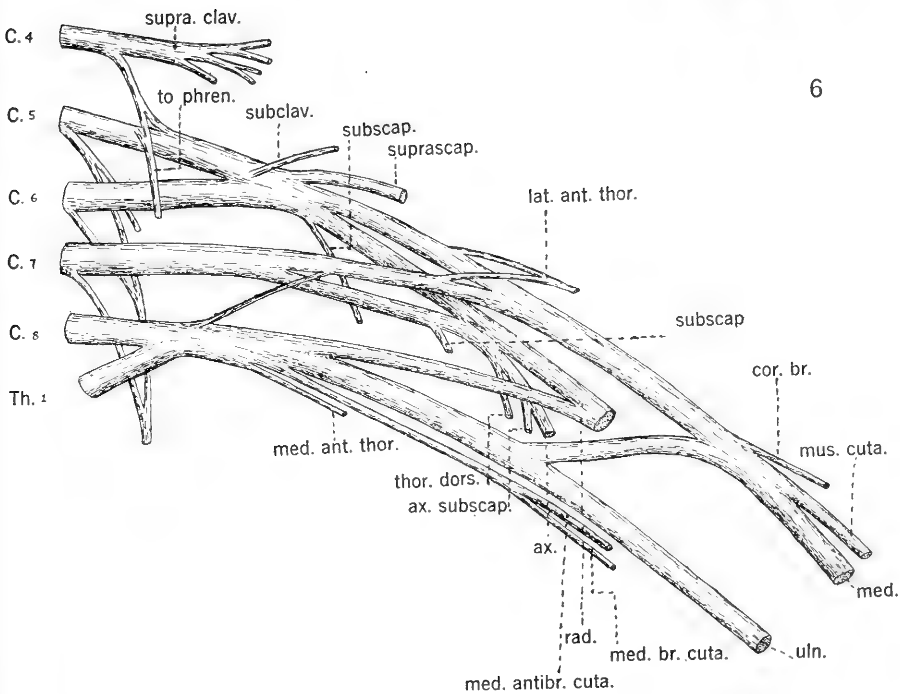

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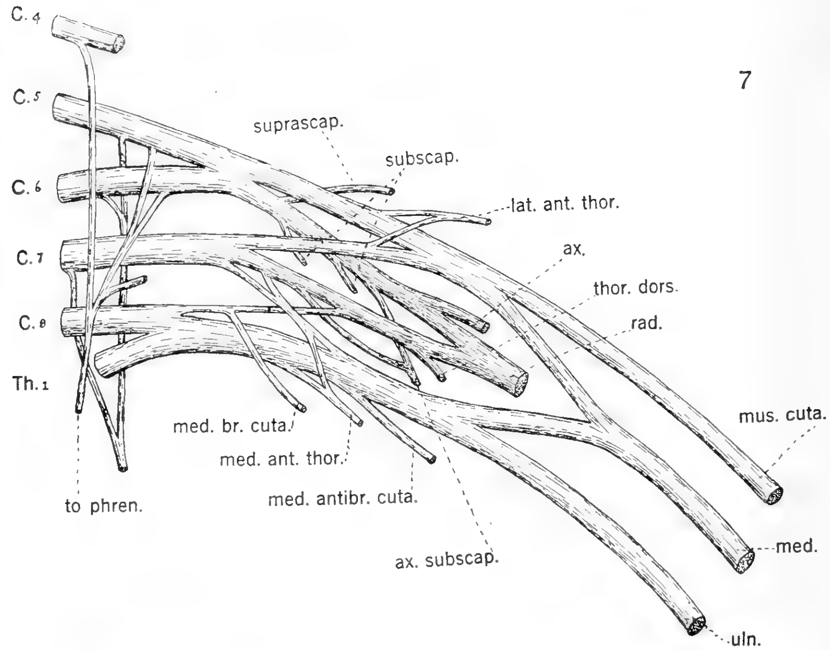
Fig. 5 From the left side of a colored female, age 14 years. Group 1, Type A.

Fig. 6 From the left side of a white male, age about 40 years. Group 1, Type C



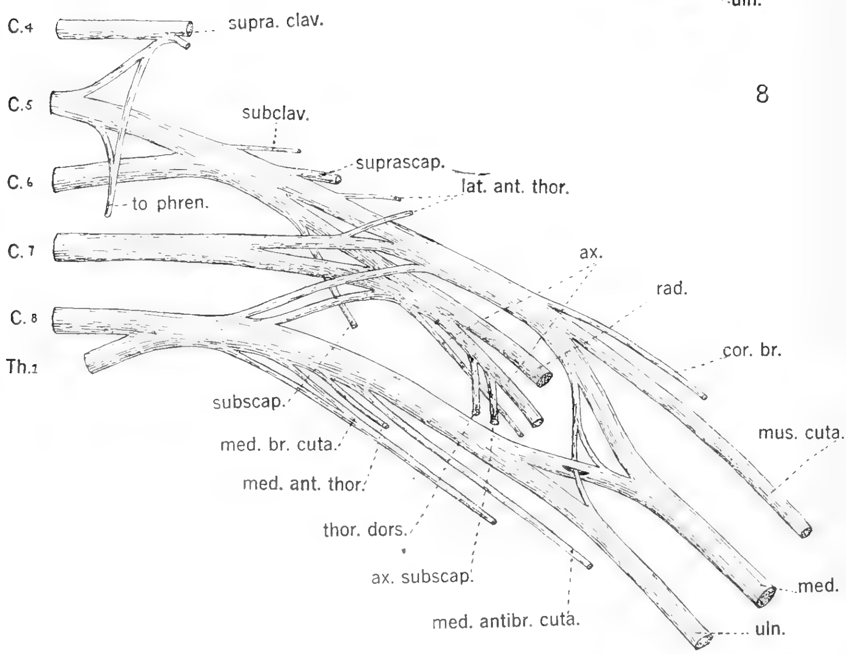


Fig. 7 From the left side of a white male. Group 2, Type D.

Fig. 8 From the left side of a colored male, age 25 to 30 years. Group 3, Type G.

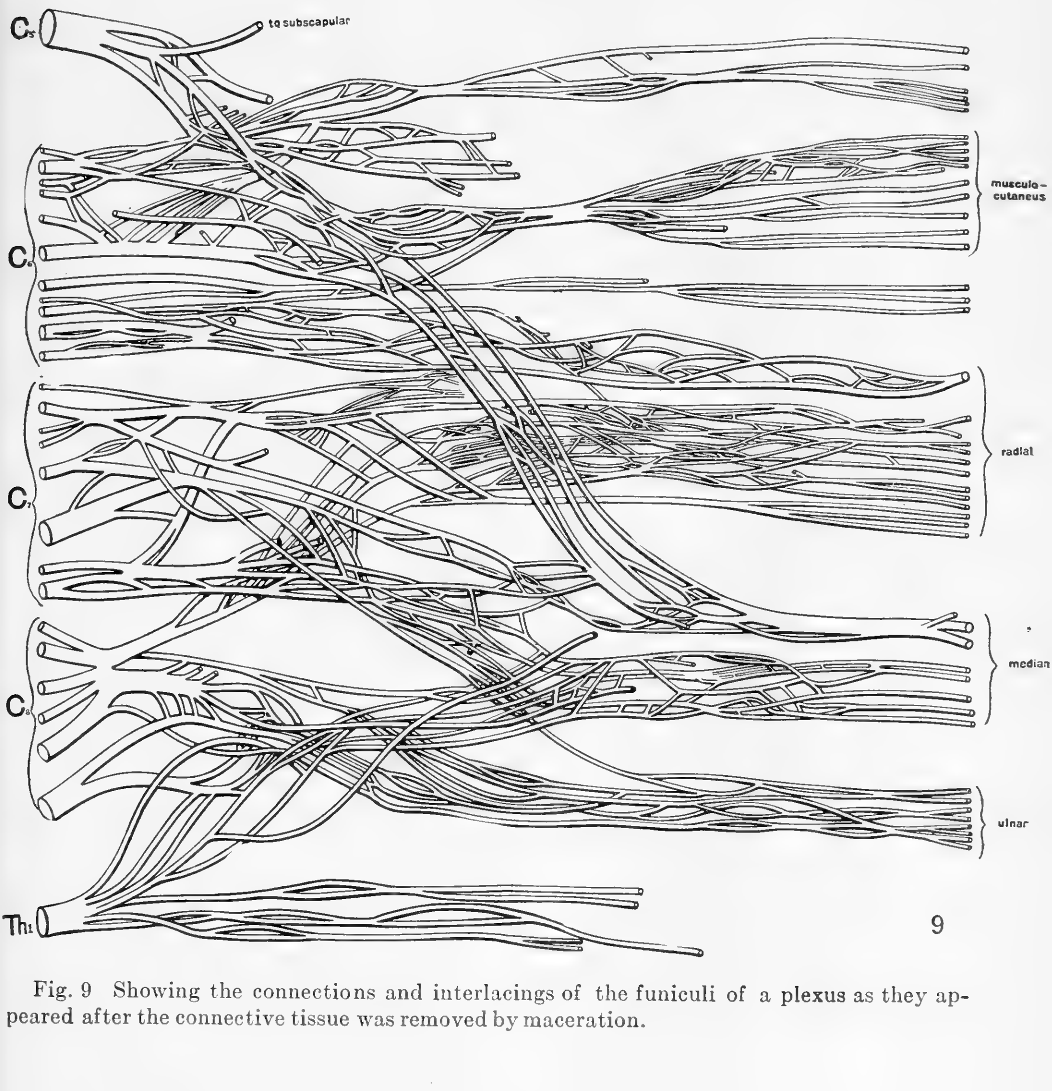
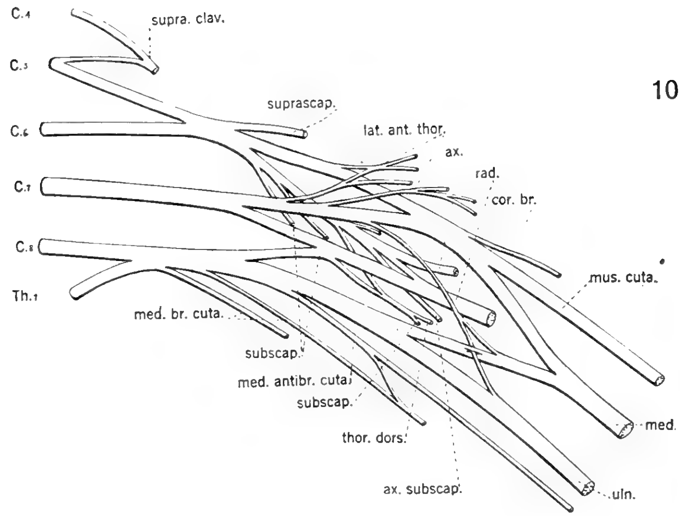


Fig. 9 Showing the connections and interlacings of the funiculi of a plexus as they appeared after the connective tissue was removed by maceration.



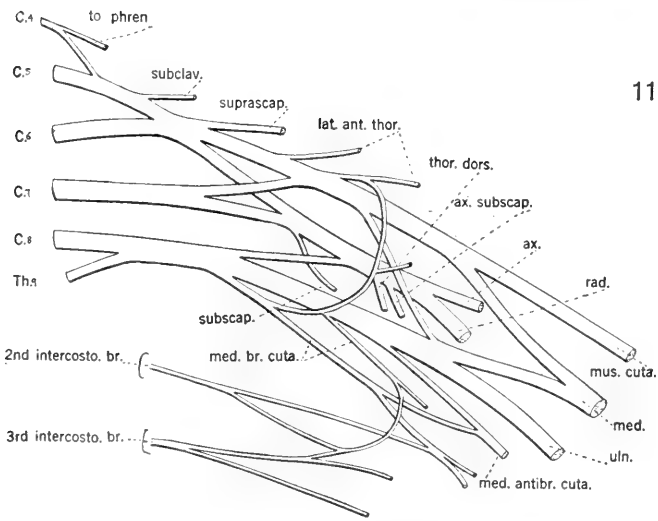
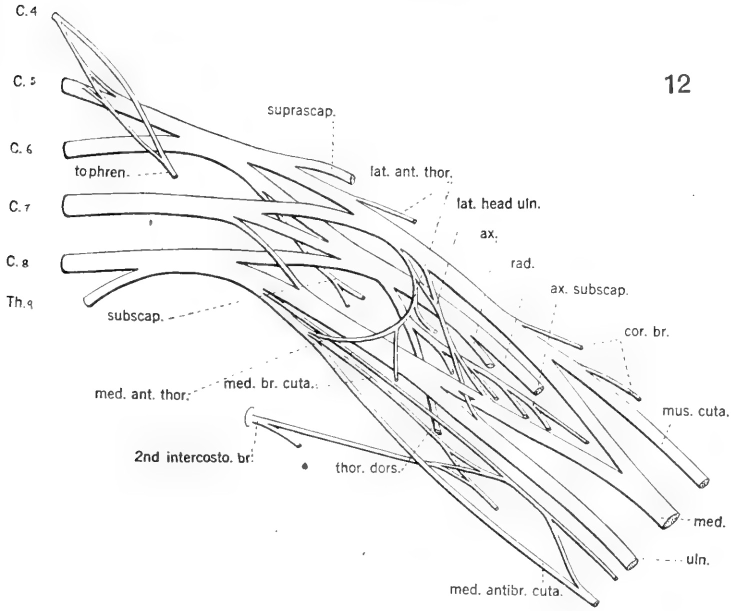


Fig. 10 From the left side of a white male, age 35 years. Group 2, Type F.
Fig. 11 From the left side of a colored female, Group 1, Type B.



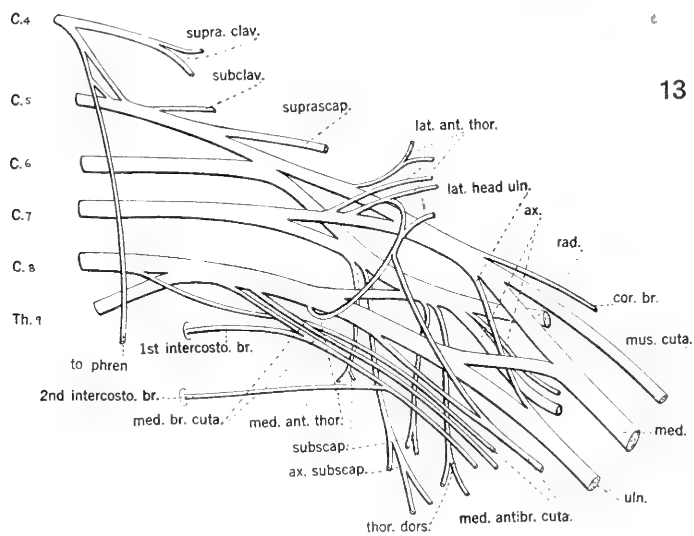
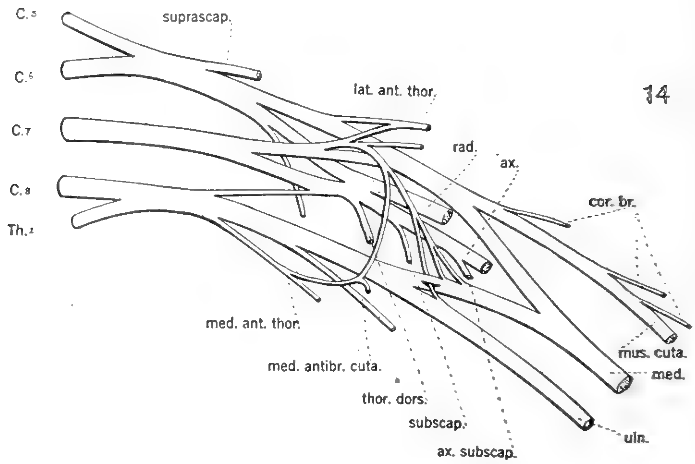


Fig. 12 From the right side of a colored female, age 25 to 30 years, reversed. Group 1, Type A.

Fig. 13 From the right side of a colored female, age 30 years, reversed. Group 1, Type B.



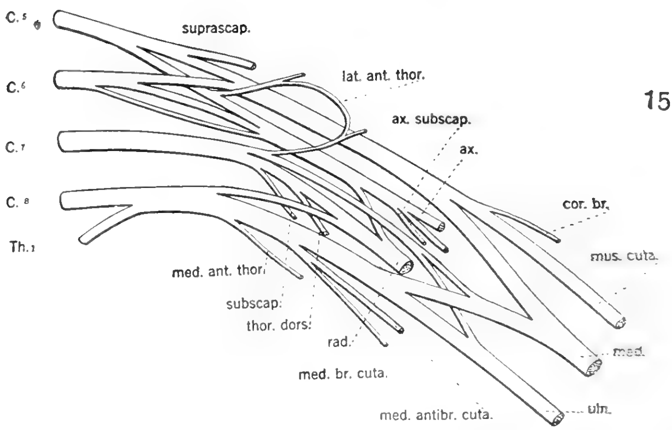


Fig. 14 From the left side of a white female, age 61 years. Group 2, Type E.

Fig. 15 From the right side of a white male, age 60 years, reversed. Group 2, Type E.

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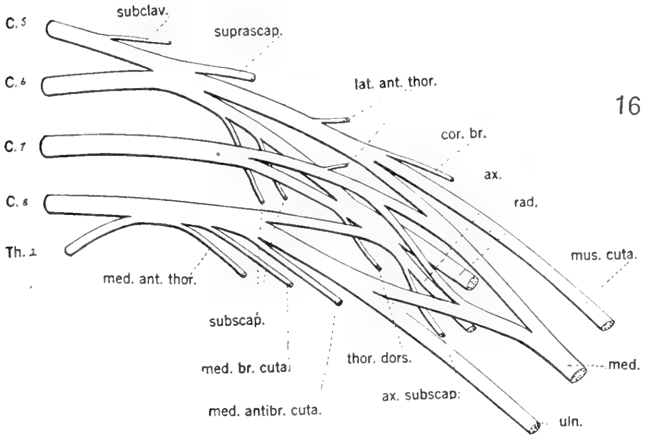

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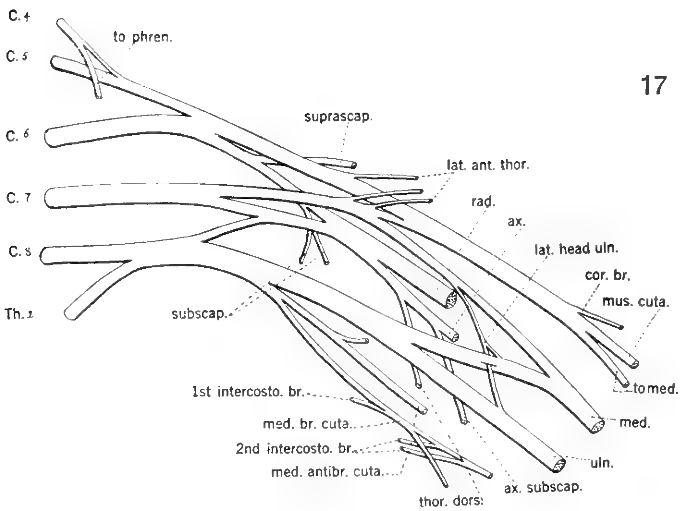
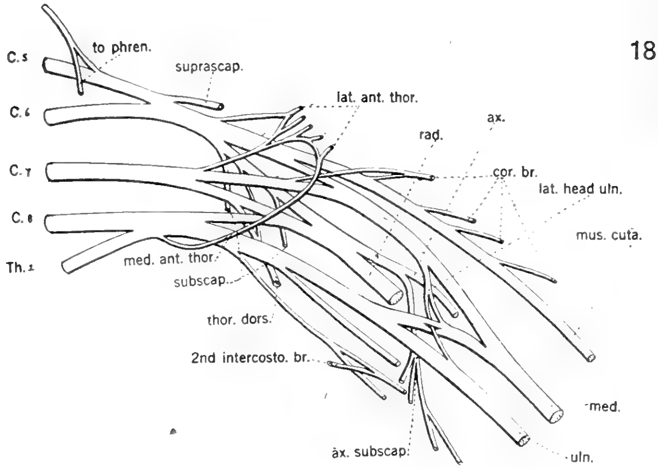


Fig. 16 From the right side of a white male, age 55 years, reversed. Group 2, Type E.

Fig. 17 From the left side of a colored male, age 20 to 25 years. Group 1, Type B.



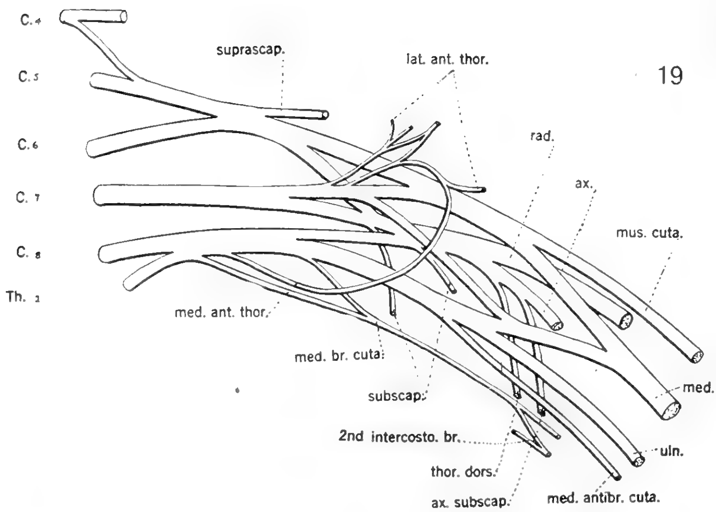
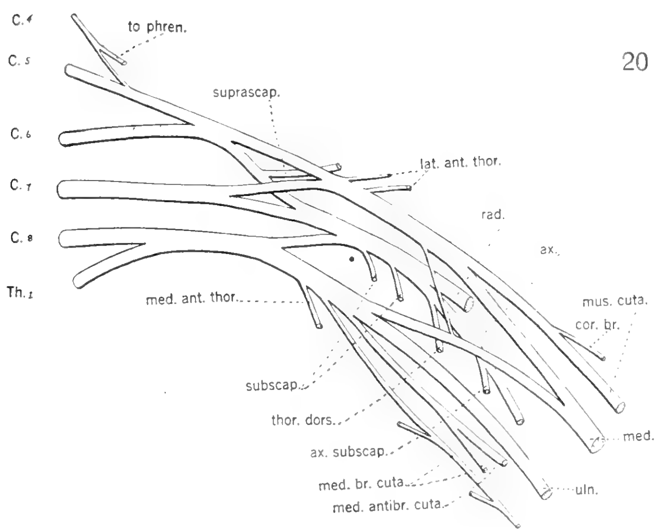


Fig. 18 From the right side of a colored male, age 20 to 25 years, reversed. Group 1, Type B.

Fig. 19 From the right side of a white male, age 55 to 60 years, reversed. Group 1, Type B.



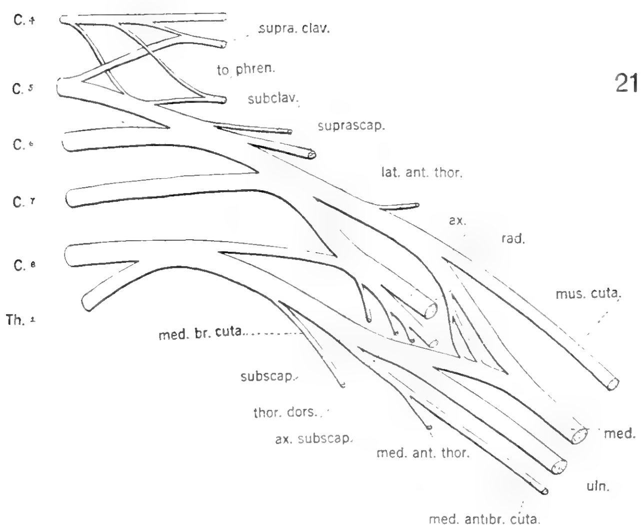
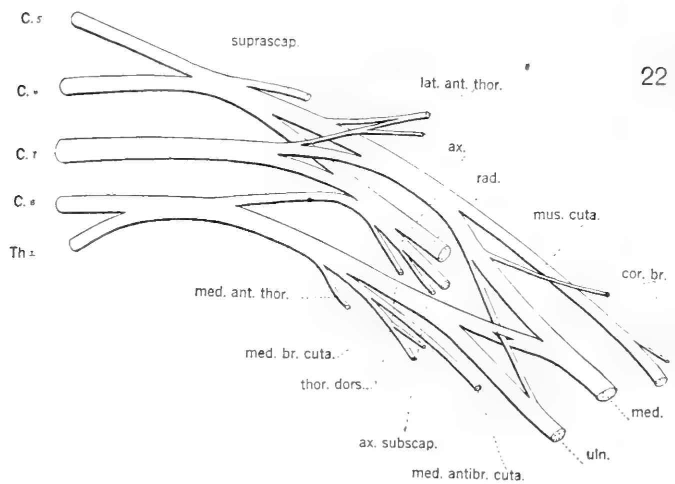


Fig. 20 From the right side of a white male, age about 40 years, reversed. Group 1, Type B.

Fig. 21 From the left side of a white male, age about 70 years. Group 2, Type F.



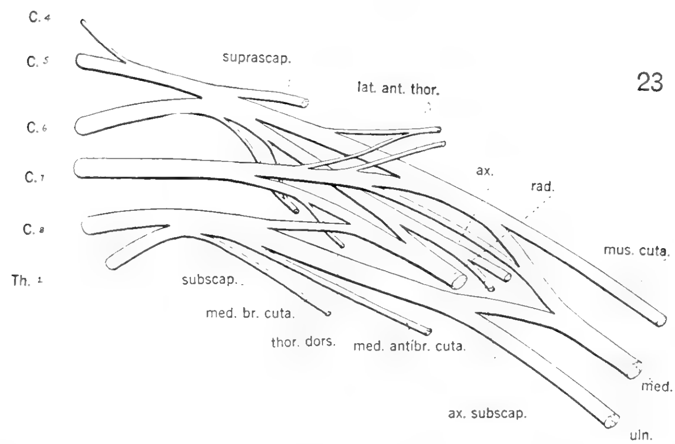
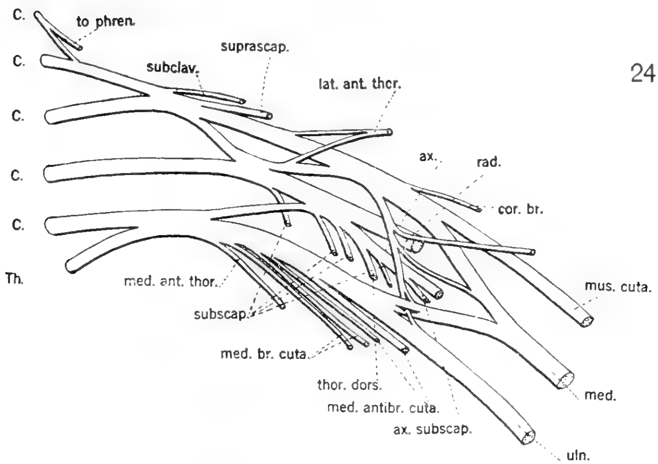


Fig. 22 From the right side of a white male, age 38 years, reversed. Group 2, Type E.

Fig. 23 From the right side of a colored male, age 45 years, reversed. Group 1. Type B.



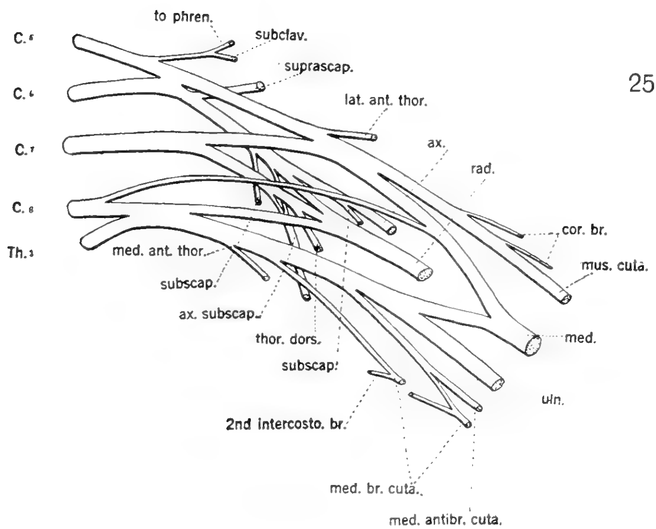


Fig. 24 From the right side of a colored male, age about 25 years, reversed. Group 1, Type B.

Fig. 25 From the right side of a colored male, age 35 years, reversed. Group 2, Type E.

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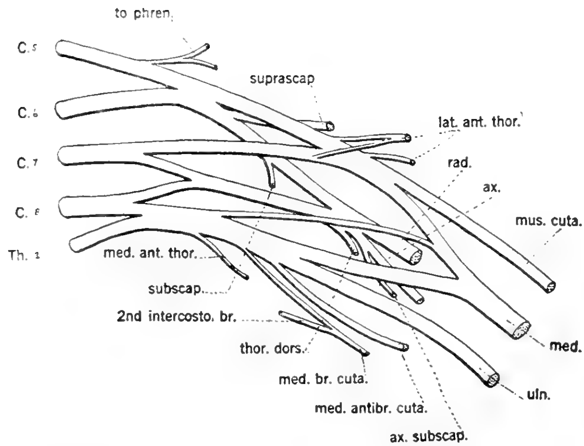

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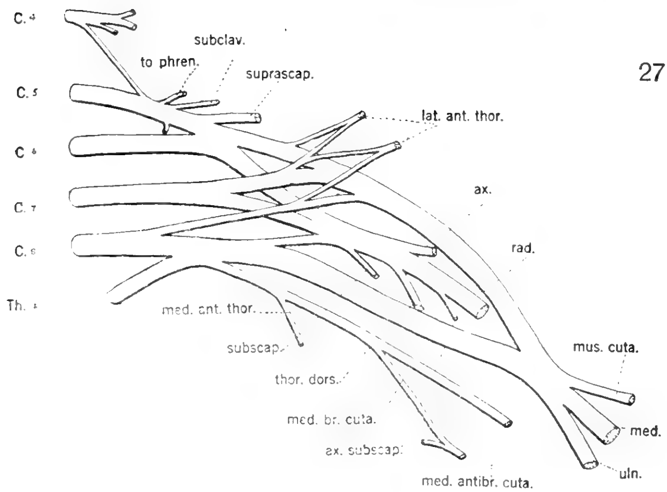
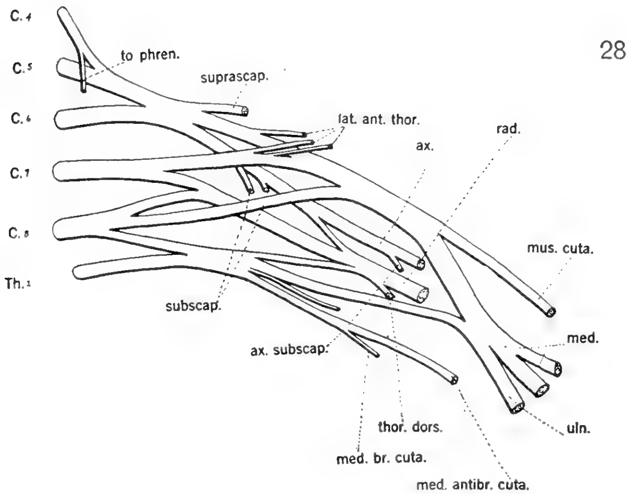


Fig. 26 From the right side of a colored male, reversed. Group 2, Type E.

Fig. 27 From the right side of a colored male, age 40 years, reversed. Group 1, Type C.



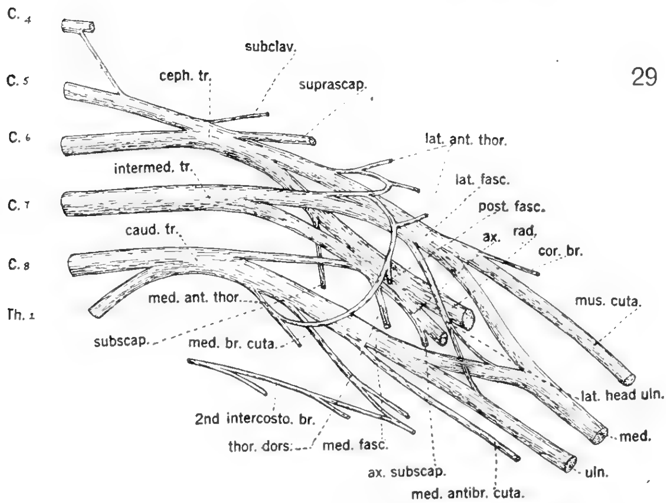


Fig. 28 From the left side of a colored male, age 40 years. Group 1, Type C.
Fig. 29 A composite, typical plexus.

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ON THE AGE OF HUMAN EMBRYOS

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TWO FIGURES AND EIGHT TABLES

In the Manual of Human Embryology, published seven years ago, I presented the evidence by which we may determine the age of an embryo or fetus, in my chapter dealing with this subject.[1] It was there pointed out that the best check in arranging embryos in time sequence is obtained from our knowledge of comparative embryology; also, that the only factor which can be depended upon in every case is what I then termed the 'menstrual age;' that is, the age of the embryo as computed by the time elapsing between the beginning of the last menstrual period and the date of the abortion.

In order to procure a satisfactory curve of growth for the whole period of gestation, I succeeded in collecting about 1000 specimens from the different months of pregnancy with the data given concerning them; namely, the measurements of the embryos and the dates of menstruation and of abortion. It was also necessary to establish standard measurements for the embryos, chief of which are sitting-height and standing-height;

[1] Mall, Franklin P. 1910 Determination of the age of human embryos and fetuses. Manual of Human Embryology, Chap. 8. Edited by Franz Keibel and Franklin P. Mall, Philadelphia; German edition, Leipzig, 1910.

1903 See also Note on the collection of human embryos in the Anatomical Laboratory of the Johns Hopkins University. Johns Hopkins Hospital Bulletin, vol. 14.

In the second paper I gave a formula by which the age of embryos up to 100 mm. long could be determined. That is, to multiply the CR length in millimeters by 100 and extract the square root; the product is the age in days. I wish to state that this formula gives the age according to the His convention, which I now believe to be incorrect, as demonstrated in my chapter in the Manual. This conclusion was also reached independently by Bryce and Teacher.

A fairly complete bibliography is to be found in the papers by Mall (1), Bryce and Teacher (3), Triepel (2) and Grosser (6).

these are known respectively as crown-rump (*CR*) and crown-heel (*CH*). Tables were prepared by which the average measurements of the one for a given stage could be converted into the average measurements of the other; for it is well known that embryologists are given to using the crown-rump measurement for smaller specimens, while anthropologists and obstetricians generally use the crown-heel measurement for larger specimens.

MENSTRUATION AGE

My tabulation of the menstrual age was made as follows: All the measurements of the embryos and fetuses were converted into crown-rump or sitting-height measurements. These were then used as ordinates, while the menstrual ages were used as abscissae; in other words, each specimen was entered upon a chart in which the menstrual age and the sitting-height together made a co-ordinate. In this way the 1000 specimens were spread over a millimeter chart, 500 mm. high and 350 mm. wide. It was found that the individual records arranged themselves along a path about 20 mm. wide at the base line, and about 40 mm. wide toward the upper margin of the chart. In addition to this central zone containing most of the records there were numerous scattered entries far out of line. These were especially numerous at the bottom of the chart, which would indicate that in early abortions there is an undue number of poor records; or, at least, records showing greater irregularity in the menstrual periods. In order to determine a mean menstrual age the chart was marked square by square in such a way that exactly one-half of the records were circumscribed by two lines enclosing the usual or normal cases, leaving one quarter of the scattered records to the right of one line, the other quarter to the left of the other line. The first group includes those specimens which grew very slowly and may have been pathological; the second, those cases in which menstruation continued after pregnancy. The two lines which include the middle group are practically parallel, beginning about 20 mm. apart, around the records of the early specimens, and ending about 40 or 50 mm. apart around the specimens from the latter part of pregnancy. The distance

between these two lines was then divided exactly, and it is this line which marks the mean menstrual age of embryos throughout pregnancy. In a general way it is reproduced as the line *CH* in figure 145 in the Manual of Human Embryology (p. 200).

I have spoken of the age thus determined at different times as the menstrual age, or more properly speaking the mean menstrual age, because there is a very large probable variation. For instance, a number selected at random from the table on page 199 of the Manual, with a mean menstrual age of 51 days, would also show a probable deviation of from 40 to 62 days. Such embryos have a height of 11 mm.; therefore, when we obtain embryos of this length, we may expect that one-half of them have a menstrual age of from 40 to 62 days; in other words, in small specimens there is a probable variation of three weeks. Viewed from another angle, one-half of the embryos with a menstrual age of 51 days would range from 4 to 25 mm., while the average would be 11 mm.; hence, we are probably dealing with a pretty large error which cannot be definitely located. At present it would appear that pregnancy may begin at any time during the intermenstrual period, but it is difficult to determine the most probable time. What I published in the Manual has received careful criticism from Triepel,[2] but he nevertheless also accepts the term *menstrual age,* and recommends that we use it in the future.

COPULATION AGE

After constructing the curve and table referred to above, showing the mean menstrual age, I entered as the probable or true age a line in the curve and a column in the table which fall in a position exactly ten days earlier than the mean menstrual age. This was done for the following reasons: According to the more recent statistics of Issmer, that writer found the average duration of pregnancy in 1220 cases to be 280 days, when estimated from the first day of the last menstrual period; and in 628 cases, 269 days when estimated from fruitful copulation. In general these figures correspond with those of Ahlfeld, Hecker

[2] Triepel, A. 1915 Alterbestimmung bei menschlichen Embryonen. Anat. Anz., Bd. 46, 1914. Also Bd. 48.

and Hasler, who collected about 500 cases in which the date of fruitful copulation was given. Therefore, in a group of 1200 cases the duration of pregnancy, when reckoned from the last menstrual period, was fully ten days longer than when computed from the time of copulation; and it seems to me that in order to determine the true age it is necessary to deduct these ten days from the menstrual age. Even then I believe we should be careful not to use the word *true,* since the time of copulation does not necessarily record the time of fertilization. For this reason it might be well if we introduced the term, *copulation age,* to distinguish it from menstrual age, and from two other ages I am about to give. These could be termed *ovulation age* and *fertilization age,* the latter being the only true age, since we must always figure the beginning of development from the time of fertilization.

The curve in the chart, which I gave in my publication, as representing the true age, but which I now will speak of as the copulation age, was constructed from cases of newborn children, and is probably the more valuable because it eliminates all of the irregularities of early pregnancy which accompany natural abortion. After the curve was completed, however, we received into our collection a few embryos, measuring less than 25 mm., the accompanying records of which gave the time of copulation as well as of menstruation. The copulation ages of these specimens were then entered upon the chart shown in the Manual with stars (fig. 147), and curiously enough nearly all of them fall exactly upon the line of the curve, showing that what was assumed to be a difference at the end of pregnancy is also indicated again in specimens from the beginning of pregnancy. In both cases the difference between the menstrual age and the copulation age is about 10 days. When this chart was made it contained seven stars, but after it had been sent to the printer I found another case in the literature. Also, about the same time I received a copy of a book published by Bryce and Teacher,[3] which gave a second case, and these were added to the curve.

[3] Bryce and Teacher 1908 Contributions to the study of the early development and imbedding of the human ovum. Glasgow.

To my great surprise and pleasure I found that these authors had reached a conclusion similar to mine; namely, that the age of young embryos is no longer to be computed according to the convention of His. They not only give a detailed and excellent account of their own specimen, but also reconsider all other cases of young specimens in relation to their age, which have been published by well-known writers. They assume that the copulation age is probably very nearly the true age of embryos, and that henceforth we will have to consider the question from this standpoint.

According to Bryce and Teacher, it is now generally admitted that the menstrual cycle in man and monkeys is homologous with the oestrus cycle of the lower mammals. The oestrus cycle is divided by Heape into pro-oestrum, oestrus and dioestrum, and this division has been confirmed for many mammals by his own researches and those of F. H. A. Marshall. During pro-oestrum the generative organs of the female show signs of special activity, such as swelling of the vulva, coloration or flushing of the surroundings, and a discharge of blood or mucus from the vagina. This is immediately followed by the 'oestrus,' or 'period of desire,' during which alone the female is capable of impregnation and will receive the male. If pregnancy does not occur, oestrus, after a brief space in which desire subsides (metoestrum), is succeeded by a period of quiescence or dioestrum, which lasts till pro-oestrum again sets in. In polyoestrous mammals several cycles of this kind may follow one another. Menstruation in the human female is homologous with pro-oestrum, as first pointed out by Heape. Though there is no fixed 'period of desire' there is an indication that a vestige of this persists, in the fact that a phase of more pronounced oestrus commonly succeeds the cessation of menstruation. This view is confirmed by our records, for we frequently hear from a patient that a fruitful copulation occurred shortly after the menstrual period; and it may be that this opinion records also the rupture of the Graafian

follicle. According to J. G. Clark[4] this is accompanied by vascular hyperemia of the ovary, and the possibility of a spasm of the ovary is not to be excluded, for there is an abundance of muscle in this organ which no doubt has a function to perform.

The following histories include all cases from our collection in which the copulation history is given. I have also added the Watt[5] case because it is the only one I have been able to find in the literature since the publication of the Manual. I have included all cases because I think it is best not to select those which suit my convenience in making a curve, but to give the poor material together with the good. A few of the records are sufficiently complete to be unimpeachable; the remainder are given for what they are worth.

No. 1399

(Dr. H. N. Mateer, Wooster, Ohio.)
Embryo, GL 1 mm. Chorion 10 x 9 mm. From a hysterectomy. Copulation September 19 and September 27. Operation, October 19. (I have been unable to find out date of last period, but it is probably recorded.) Copulation age 22 or 30 days. If the former is taken, it matches the curve exactly.

No. 779

(Dr.————, Baltimore.)
Embryo, GL 2.75 mm. The specimen though otherwise normal was later found to have spina bifida. It came from the physician's wife. She is 37 years old, and is the mother of one child and this is her first abortion. She is very anxious to have children. Last period, August 29 to September 2. Abortion, October 12. Fruitful copulation, in the woman's opinion, September 25 and later. She does not state specifically that copulation occurred between September 2 and September 25. Menstrual age, 44 days. Copulation age, 17 days or less. Doubtful case.

[4] Clark, J. G. 1899 The origin, growth and fate of the corpus luteum as observed in the ovary of the pig and man. Johns Hopkins Hospital Reports, vol. 7.

1900 The origin, development and degeneration of the blood-vessels of the human ovary. Johns Hopkins Hospital Reports, vol. 9.

[5] Watt, J. B. 1915 Description of two twin human embryos with 17 to 19 paired somites. Contributions to Embryology, vol. 2, Carnegie Institution of Washington, Publication No. 222.

Watt's case

(Dr. Watt, Toronto.)

Twin embryos, GL 2.75 and 3.55 mm.

Mother, a German Jewess, 30 years old, robust and healthy, four children and this one abortion. Last period, December 3 to 6, 1907; first copulation December 20. Slight flow January 3, with similar flow on January 11, 12 and 13, abortion following on January 14. Menstruation age, 42 days. Copulation age, 25 days or less.

No. 1182 b

(Dr. C. E. Caswell, Wichita, Kansas.)

Woman aged 27, four living children and one abortion. Husband has syphilis and so has one child. Mother seems to have escaped. Last period, March 25 to April 4. Abortion, May 10. Mother is sure that conception took place April 14. Menstrual age 46 days. Copulation age, 26 days. Doubtful case.

No. 470

(Dr. H. C. Ellis, Elkton, Md.)

Embryo, CR 4 mm. Chorion, 20 x 13 mm.

Mother, 24 years old, two healthy children. Abortion during an attack of mumps with very high fever. Last period October 5, 1910, and copulation about October 15. Abortion November 9. Menstrual age, 35 days; copulation age not over 25 days.

No. 588

(Dr. G. L. Wilkins, Baltimore.)

Embryo, CR 4 mm.

Last period January 26 to February 3. Had no intercourse with husband for several weeks prior to this and only three or four days after period but not later. Abortion March 16, 1912. She has had two healthy children, 14 and 20 years old respectively, and not less than eleven abortions. Dr. Wilkins believes that all the abortions were induced. Menstrual age, 50 days. Copulation age, 38 or 39 days.

No 1507

(Dr. C. B. Ingraham, Denver, Colorado.)

Macerated embryo, GL 4 mm.

A Jewess who last menstruated May 7 to 11; abortion June 22. Woman had opportunity to become pregnant shortly after this period or again just before the next. Menstrual age, 46 days; copulation age, 40 days or 17 days. Record not satisfactory, especially since specimen s also pathological.

No. 208

(Dr. J. Y. Dale, Lamont, Pa.)

Normal embryo, CR 7 mm., GL 8 mm. The specimen was enclosed in an almond-shaped ovum, measuring 22 x 11 x 11 mm., and there was considerable magma within the exocoelom. The specimen came from a married woman whose last period began on December 28, 1901, and who had coitus only twice, January 5 and January 7, between this period and the time of abortion, February 15, 1902. Dr. Dale informs me that the data are entirely reliable, as both the woman and her husband are thoroughly trustworthy. The specimen was secured for me by Prof. John G. Clark of the University of Pennsylvania, who thought that its unique history gave it greater value. Menstrual age, 49 days; copulation age, 39 to 41 days.

No. 1461

(Dr. H. A. Wright, Seattle, Washington.)

Embryo, CR 9.8 mm.

Menstrual age, 28 days; copulation, 27 days. Data inaccurate and incomplete. Not a reliable case.

No. 443

(Dr. William Grant, Baltimore.)

Embryo about 10.5 mm. long.

The specimen was sent at the suggestion of Prof. T. S. Cullen on account of its interesting history. Menstrual age, 27 days and copulation age, 28 days. On account of the manner in which the history was given, and because of the degree of development of the embryo, the data can hardly be admitted as correct. The husband had been away from home for four months prior to the time of coitus, which was on the last day of menstruation. The woman is the mother of four healthy children and menstruates regularly every 28 days. The patient was reluctant to show the specimen to the physician, and both she and her family defended her character, a fact which would seem still further to convict her. For this reason the record is not to be considered reliable so far as the age of the embryo is concerned.

No. 167

(Dr. A. H. Ritter, Brooklyn, N. Y.)

Embryo, CR 14.5 mm., NL 13.5 mm.

The normal embryo was sent in a beautiful normal ovum measuring 30 x 30 x 20 mm. The specimen came from a multipara whose last period was from November 26 to December 2, 1899. First copulation

after the period on December 15. Due to a surgical operation on January 24 there was continuous hemorrhage until January 3 when the ovum was passed. In the event that conception took place after the last period, this specimen could not be more than 46 days old. Menstrual age, 65 days; copulation age, 46 days.

No. 1390

Dr. G. N. J. Sommer, Trenton, N. J.)

Embryo CR 18 mm.

Last period, December 18 to 22. Operation for tubal pregnancy, February 10. Conception took place on December 25, as the woman was in the habit of using preventive means and same were not used on Christmas eve. Menstrual age, 54 days; copulation age, 47 days. Reliable case.

No. 1584

(Dr. F. H. Church, Bonnville, N. Y.)

Embryo, CR 18 mm. Chorion 35 x 31 x 25 mm.

Unmarried woman, age 21, first pregnancy. Last period August 15; menstruation regular, every 28 days. Criminal abortion, October 10. Coitus, September 13 and 14. Menstrual age, 56 days; copulation age, 26 and 27 days. Not reliable.

No. 26.

(Dr. C. E. Simon, Baltimore.)

Fetus, CR 25 mm., CH 30 mm. This specimen, which was somewhat injured and therefore difficult to measure with precision, was brought to me by Dr. Simon, February 25 (?) 1894 with the following history. The mother, an unmarried woman 27 years old, was a servant in Dr. Simon's family, and had but recently come from Germany. She remained at home continually until New Year's eve, when she went to a ball and remained out all night. Her last period took place on December 12 and lasted six days. During the night of December 31 she was with her lover and the abortion followed on February 25. On January 16, after missing her January period, she took a cupful of mustard powder with the hope that it would produce abortion, but instead it nearly killed her. On January 21, she recovered, and resumed her household duties. (See record of the case by Simon, N. Y. Med. Jour., March 17, 1894.) Later she fell into the hands of an abortionist and the embryo came away during the night of February 24. Dr. Simon assured me at the time of the abortion that it was absolutely impossible for the pregnancy to have taken place at any time excepting the night of December 31.

Menstrual age, 75 days; copulation age, 56 days. Reliable case.

No. 616

(Dr. S. P. Warren, Portland, Me.)

Embryo, CR 26 mm. Unmarried woman, 18 years old; last period began August 9 and continued 7 days. Coitus three times within 9 days after the last period. Curetted after 24 hours of pain, October 13, 1912.

Menstrual age, 65 days; copulation age, 56 days (?). Not reliable.

No. 1535

(Dr. Philip F. Williams, Philadelphia).

Embryo, CR 28 mm. Chorion, 50 x 45 x 15 mm.

Unmarried woman, 20 years old; first pregnancy. Last period, May 3 to 7. Abortion, July 6. Last coitus, May 10 (?). Menstrual age, 62 days; copulation age, 55 days. Doubtful age, but it falls close to the time of the probable age.

No. 373

(Specimen loaned by Prof. Simon H. Gage, Ithaca, N. Y., Cornell Collection, Homo No. 11.)

Embryo, CR 31 mm.

Last period, May 9. Conception, May 21. Natural miscarriage July 17, after 2 to 3 days bleeding. No other data. According to these records the menstrual age is 69 days and the copulation age 57 days.

No. 849

(Dr. Shipley, Baltimore.)

Embryo, CR 52.5 mm.

Mother, white, unmarried, age 20. Last period, December 11 to 15, 1913. Abortion, March 3. Coitus from which mother dates pregnancy occurred just after the cessation of the last menstrual period in December, but she admits that she had frequent intercourse previous to this period.

Menstrual age, 82 days; copulation age, 77 days (?) Doubtful case.

No. 591

(Dr. G. C. McCormick, Sparrows Point, Md.)

Embryo, CR 62 mm. End of last period, January 1, 1912. Coitus, January 7; abortion, March 29.

Menstrual age, 93 days; copulation age, not over 82 days.

No. 1635

(Dr. Henry Leaman, Philadelphia.)

Embryo, CR 70.5 mm. Mother, 40 years old, 9 children. Last

period, August 26 to 30; abortion, November 29. Copulation but once about September 3 to 15. Self induced abortion.

Menstrual age, 95 days; copulation age, 75 to 87 days. Records contradictory.

No. 322

(Dr. West, Bellaire, Ohio.)

Embryo, CR 85–90 mm.

The specimen is probably from an induced abortion. The mother says that fruitful coitus took place on June 17 and the abortion on September 20, copulation age 95 days.

No. 1295 c

(Dr. L. J. Commiskey, Brooklyn, N. Y.)

Embryo, CR 87.

Woman, 43 years old, mother of one child and this is her second abortion. Last period, April 24 to 28; abortion, July 27. Woman states with great certainty that the productive coitus took place either May 4 or 6.

Menstrual age, 94 days; copulation age, 84 or 82 days. Doubtful case.

No. 1310

(Dr. B. G. Pool, Washington, D. C.)

Embryo, CR 95 mm.

First pregnancy of unmarried woman, 18 years old. Last period, July 25 to 30, 1915; abortion, November 6. Said to be from a single coitus on August 9. Menstrual age, 104 days; copulation age, 89 days. Doubtful case.

No. 894

(Dr. E. L. Mortimer, Baltimore, Md.)

Embryo, CR 121 mm.

White mother, age 29, three children and two abortions. Last period, July 24 to 28; criminal abortion, November 21. Husband works on a boat and returned home August 1.

Menstrual age, 120 days; copulation age, not over 112 days.

No. 142

(Dr. G. H. Hocking, Govans, Md.)

Embryo, CR 142 mm.

Mother, age 43, has five children. Menstruated May 29 to June 9; abortion, October 5 after several weeks' flow. Woman says pregnancy could not have taken place before June 18.

Menstrual age, 129 days; copulation age, 109 days. Doubtful case.

The summary of these cases together with all others of the same kind which I have been able to gather from the literature, is given in table 1. This is an elaboration of the table given in the Manual. The data are sufficiently complete so that those who choose may look up the original records. Most of them, however, will be found in abstract form in the articles by Triepel and by Grosser.[6] The specimens in the Carnegie Collection are recorded above.

All of the specimens just given are entered upon figure 1. The mean menstrual age and the mean copulation age are taken from the data given in the table and in the curve published in the Manual. For the specimens here considered the menstrual ages are indicated by means of dots, the copulation ages by large solid circles. The time is calculated by days, and the measurements of the embryos are crown-rump. The numbers of several specimens for which the copulation age is given are marked in figure I; for instance, No. 443 and No. 1310. One of the Rabl cases is also indicated. I am of the opinion that all these marked records should really be excluded from the figure as they do not appear to be very reliable. However, I have included them for the sake of completion. Six of the copulation cases are crossed in the figure with an X, and are given again in table 2.

It will be noticed that these six records fall almost exactly upon the curve given. They are, I believe, the only ones which are entirely reliable; that is, they record embryos which are the product of single copulations, and for this reason their maximum ages are established. A word regarding specimen No. 26, which is recorded in the literature as representing an embryo 30 mm. long. As we are at present dealing with CR measurements, this should be 25 mm. It appears on the chart in the Manual as 30 mm., for the reason that the curve was constructed on the basis of the standing height, or CH length of the embryos. The tables given by Triepel and by Grosser should, therefore, have this measurement corrected accordingly.

I have also entered upon my figure the ages of the embryos

[6] Grosser, O. 1914 Alterbestimmung junger menschlichen Embryonen; Ovulations und Menstruationstermin. Anat. Anz., Bd. 47.

TABLE 1

LENGTH OF EMBRYO		MENSTRUAL AGE	POSSIBLE TIME OF COPULATION IN DAYS BEFORE ABORTION	AUTHOR
	mm.	*days*		
Embryo	0.15	38	Exactly 16 days	Bryce-Teacher, 1908
Ovum	5.5 x 3.3	42	20 days before and earlier	Reichert, 1873
Embryo	1.0		22 and 30 days before	No. 1399
	1.2 (?)	38	19 days (Delaporte)	See Grosser Anat. Anz. xlvii, 1914
	1.3	34	Exactly 21 days	Eternod, Anat. Anz. xv, 1899
	1.5 (?)	35	14 days	Fetzer, Anat. Anz. Erg. Hft. xxxvii, 1910
	2.75	44	17 days and later	No. 779
	2.75	42	25 days and later	Watt, Carnegie Contributions to Embryology, ii, 1915
	3.33	42	(Twin)	
	3	46	26 days (?)	No. 1182b.
	3.2	48	40 days and later	His, AME., vol. 2, 1882
	4.0	35	25 days (?)	No. 470
	4.0	50	38 days	No. 588
	4.0	46	40 days and 17 days (?)	No. 1507
	6.0	50	40 days and later	Kollmann's Atlas, 1907
	7	49	39 and 41 days	No. 208
	7.75	57	45 days and later	His
	8.8	42	Exactly 38 days	Tandler, Anat. Anz., xxi, 1907
	9.8	28	27 days (?)	No. 1461
	10	60	49 days and earlier	His
	10.5	27	22 days (?)	No. 443
	11	55	31 days (?)	Rahl, Entwickl d. Gesicht
	13.6	63	53 days and later	His
	14	65	Exactly 47 days	Rahl
	14.5	65	46 days and later	No. 167
	18	54	Exactly 47 days	No. 1390
	18	56	26 or 27 days (?)	No. 1584
	25	75	Exactly 56 days	No. 26
	26	65	56 days	No. 616
	28	62	55 days (?)	No. 1535
	31	69	57 days	No. 373
	52.5	82	77 days (?)	No. 849
	62	93	Not over 82 days	No. 591
	70.5	95	75 or 87 days (?)	No. 1635
	85 (?)		95 days	No. 322
	87	94	82 or 84 days (?)	No. 1295c
	95	104	89 days (?)	No. 1310
	121	120	Not over 112 days	No. 984
	142	129	Not over 109 days	No. 1284

according to their degree of development as given by Triepel in order to show that he has practically adopted the curve of development given by Bryce and Teacher and also by myself. He has really taken what I have designated as the copulation age, minus about two days for each stage, assuming, as do also

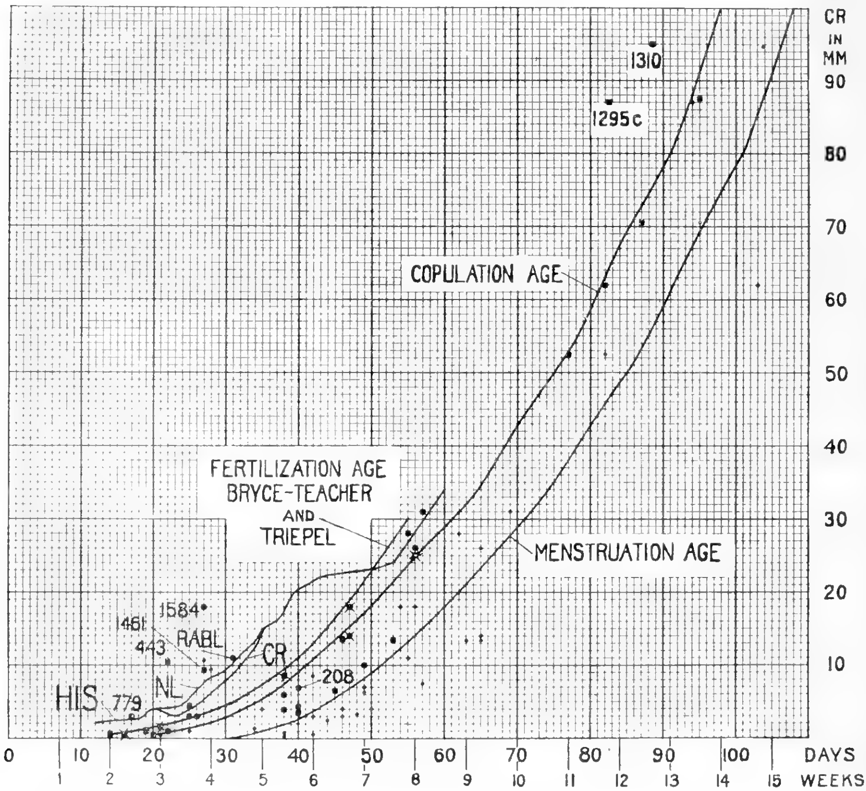

Fig. 1 Menstruation age and copulation age taken from the curve constructed by me and published in the Manual of Human Embryology. All embryos are entered with CR length. I have also added for the sake of comparison the curve giving the convention of His. NL and CR give the neck-rump and crown-rump lengths respectively, according to His. The fertilization age is according to Bryce and Teacher for smaller embryos, and according to Triepel for larger ones. The dots record the menstrual age of the embryos under consideration, and the squares the copulation age. The crossed squares mark the best records, as mentioned in the text.

It may be noted again that the curves are not constructed from these records, but the records are entered to test the curve.

TABLE 2

AUTHOR	CR LENGTH OF EMBRYO	COPULATION AGE
	mm.	*days*
Bryce and Teacher	0.15	16
Eternod	1.3	21
Tandler	8.8	38
Rabl, V.	14.0	44
No. 1390	18.0	47
No. 26	25.0	56

Bryce and Teacher, that there is this interval of two days between copulation and fertilization. For the sake of completion the curve giving the His convention is also included in the figure.

I wish again to emphasize the fact that the curves given in the figure are not constructed from the records of the specimens in question, and it is quite clear, I think, that the new cases give no reason for materially altering the mean copulation curve as given by me in the Manual seven years ago. The relation of these curves to the ovulation age and to the fertilization age remains to be established, and as far as the evidence will permit this will be done in the following paragraphs.

OVULATION AGE

The question of the time of ovulation in relation to menstruation or to copulation is by no means answered, although the literature upon the subject is extensive. If the time of ovulation could be definitely determined we would then be able to ascertain the ages of embryos with very fair precision. Wherever possible we have collected ovaries with our specimens, but so far have obtained only one accompanying a young ovum. This specimen, No. 970 in our collection, is from a Filipino girl, 16 years old, who died four days after taking hydrochloric acid with suicidal intent on account of her condition. The ovum, which measures 5 x 3 mm., is not quite normal in appearance but is well implanted. The corpus luteum is well formed, and solid, with no remnant of blood within it. The Herzog[7] specimen, which

[7] Herzog. 1909 A contribution to our knowledge of the earliest known stages of placentation and embryonic development in man. Am. Jour. Anat., vol. 9.

is also from a Filipino woman who was killed in an accident, likewise had a small ovum measuring 2.3 x 1.2 mm., well implanted in the uterus. In this case the corpus luteum was 'fresh but closed.' The well known Reichert specimen which measured 5.5 x 3.3 mm., with a copulation age of 16 days or more, has in one ovary a well-formed corpus luteum, 20 x 17.5 mm. which has within it a small cavity containing some blood. Finally, Johnstone[8] describes and pictures the corpus luteum of an ovum almost the size of Peters' specimen, which measures 13 x 10 mm. Its center is occupied by a large mass of pale, finely granular material which stained pink with eosin. The periphery is composed of a layer of lutein cells bordered on the inside by a layer of red blood corpuscles. The lutein layer, which is 8 x 10 cells deep, is crinkled, owing to papillary ingrowths of connective tissue. There is a great deal of vacuolation of the lutein cells and the whole layer is quite vascular. The specimen came from a woman, aged 29, who died suddenly, not having missed a period, nor was it suspected that she was pregnant.

A step in advance on the study of the structure of the corpus luteum was made by R. Meyer[9] in his excellent paper on the subject. He classifies its development into four stages as follows:

1. Proliferation or early hyperemic stage of the Graafian follicle with transformation of the granular cells into lutein cells.
2. Early hyperemic stage of the corpus luteum with beginning transformation into the second stage of granular metamorphosis.

The blood-vessels now permeate the layer of lutein cells.

3. Mature or blossoming stage of the corpus luteum.
4. Stage of involution.

Sometimes when the follicle ruptures it simply collapses, and hemorrhage does not always take place within it. The specimens studied by Meyer were increased in number and reported

[8] Johnstone, R. W. 1914 Contribution to the study of the early human ovum. Journal of Obstetrics and Gynaecology of the British Empire.

[9] Meyer, R. 1911 Ueber corpus luteum-Bildung beim Menschen. Archiv für Gynaekologie, Bd. 93, 1911.

in relation to the menstrual cycle by Ruge II[10] who gives the following data:

TABLE 3

STAGE	NUMBER OF SPECIMENS	TIME OF OCCURRENCE IN RELATION TO MENSTRUATION
Proliferation	10	1 to 14th day
Vascular	10	10 to 16th day
Mature	44	16 to 28th day
Involution	18	1 to 13th day

TABLE 4

STAGE	NUMBER OF SPECIMENS	TIME OF OCCURRENCE IN RELATION TO MENSTRUATION
Proliferation	10	1 to 14th day
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Mature	44	16 to 28th day
Involution	18	1 to 13th day

Ovulation occurred in the stage of proliferation, and always during the first 14 days of the period. However, this stage does not form a regular sequence of development during the first two weeks, but the specimens were of unequal development and could not be arranged in the order of time. It is impossible to determine the time elapsing between ovulation and the formation of the third stage of mature corpus luteum, but Meyer and Ruge believe that always a number of days must intervene. Finally, the stage of involution overlaps that of proliferation. At any rate the work of Meyer and Ruge demonstrates that the fresh corpus luteum as described by Fraenkel[11] appeared a number of days before he thought it did, thus completely overthrowing Triepel's assumption that the probable time of ovulation is on the 19th day. According to Ruge, it occurs sometime during the first 14 days of the menstrual month which supports the theory I am advocating.

These are all the reliable data I have been able to collect

[10] Ruge II, Carl 1913 Ueber ovulation, corpus luteum and menstruation. Archiv für Gynaekologie, Bd. 100.

[11] Fraenkel: Archiv für Gynäekologie, Bd. 91.

regarding the time of development of the corpora lutea in human beings. I had thought that it would be possible to extend the subject somewhat further if the corpus luteum in the pig could be standardized in relation to the size of the embryo found in the uterus. This work was carried through by Corner, but unfortunately does not include the earlier stages of the corpus luteum, and it is just these data that we need if we are to determine accurately the age of freshly ruptured Graafian vesicles. Corner[12] made a careful study of the histological changes in the corpus luteum of the sow for all but the earliest stages of pregnancy. He finds that the corpus luteum is already solid at 20 days, this stage being reached earlier, he believes, than in human beings where this central cavity remains longer. By the aid of refined cytological methods he recognizes seven distinct stages during pregnancy as follows:

TABLE 5

STAGES	LENGTH OF EMBRYOS	APPROXIMATE AGE
		days
1. Preparatory period	Less than 20 mm.	(?) 25
2. Exoplasmic development	(I) 20–30	25–30
3. Exoplasmic development	(II) 30–55	30–40
4. Transitory period	55–140	40–75
5. Endoplasmic development	(I) 140–170	75–105
6. Endoplasmic development	(II) 170–220	105–110
7. Retrogression	220–290	110 to term

Although this study cannot be transferred to the human directly, it at any rate suggests that the latter may be standardized. It is hoped to establish at least a relation between the early stages of the corpus luteum and the size of the ovum and embryo; and that in the course of time the age of this body may be estimated with precision.

It may be noted that Corner showed definitely that the size of the embryo found in the uterus could be estimated with con-

[12] Corner, George W. 1915 The corpus luteum of pregnancy; as it is in swine. Contributions to Embryology, vol. 2, Publication No. 223, Carnegie Institution of Washington.

siderable accuracy by the cytological condition of the lutein cells; however, all his specimens were from corpora lutea presumably a little older than the human ones mentioned above. In a measure we may fill in the gap in the earlier stages from the report by Sobotta[13] on the development of the corpus luteum in the mouse. He found in this study that during the first 24 hours after ovulation the cavity of the follicle fills with serous fluid or blood, at the time the lutein cells become cut up into compartments by the formation of connective tissue septi. This process continues during the following day or two, and finally the central cavity is nearly obliterated, containing, however, a central mucoid nucleus at the middle of the third day after ovulation.

The irregular summary from the several species is about as follows: (1) In the mouse the central cavity of the corpus luteum is obliterated about the middle of the third day after ovulation; (2) it is obliterated in human specimens accompanying ova about the size of those studied by Bryce and Teacher, and by Peters; and (3), it is obliterated in the pig considerably before the 25th day. It may also be noted that Corner states that the corpus luteum of menstruation is of irregular shape in its development, while that of pregnancy is uniform and even. He speaks of the former as if the cells were arranged like a mob, and the latter as if organized like an army.

Finally, a few words regarding Fraenkel's studies, out of which Triepel has made so much capital. According to Fraenkel, Villemin in 39 operations found no freshly ruptured follicles in the first two weeks after the menstrual period, but observed many from 12 to 14 days before it. Fraenkel himself describes hemorrhagic follicles as follows: Very fresh, fresh, quite fresh and not very fresh, showing that his average of 19 days after the last menstrual period is not the average time of ovulation, but the average of older corpora lutea in several stages of development. From a study of Fraenkel's papers it may be seen quite clearly that what he reports as fresh corpora lutea are by no means

[13] Sobotta. 1896 Ueber die Bildung des corpus luteum bei der Maus. Archiv für Mik. Anat., Bd. 47.

necessarily fresh, but may possibly vary in age fully a week. In fact he intimates that they are not all fresh, and Triepel makes a slight allowance for this reason. These papers have been carefully analyzed by Grosser, who finally reached the conclusion that ovulation does not take place on the 19th, but at the latest on the 16th day after the beginning of menstruation. This figure is not so very far from the average given in my curve; in fact it is a little more than the average age accepted by Triepel as the normal according to the degree of development of the embryo. Triepel has attempted to force a curve which runs exactly 12 days after the average menstrual age of specimens, into one which should be exactly 19 days after this curve, in order to fit Fraenkel's opinion regarding the proper time of ovulation. This of course is an impossible feat.

The conclusion to be drawn, therefore, is that we cannot possibly establish a satisfactory ovulation age of embryos from the data now at our disposal; but I believe that we have material within our reach whereby we may eventually be able to determine with greater certainty the probable time of ovulation. Before this can be done with the human, however, it will be necessary to study anew the degree of development of the corpus luteum for various days after menstruation, with new material selected from cases which are otherwise normal. This can be done in any large gynecological clinic.

FERTILIZATION AGE

According to Bryce and Teacher, the comparative infrequency of pregnancy during continuous cohabitation points to some special circumstance connected with successful impregnation. This circumstance would appear to be simultaneous ovulation and limited power of fertilization on the part of the spermatozoa. As regards the former the work of J. G. Clark is of interest. According to this writer ovulation is accompanied with hyperemia of the ovary, and, he believes, is hastened by it. He injected the blood vessels of an ovary in which there were fresh corpora lutea, as well as swollen Graafian follicles, and found that

the injected fluid immediately ran out of the ruptured follicle. In a few instances the fluid entered mature follicles, causing them to become dense and finally to rupture when the vascular pressure was continued for a sufficiently long period. This suggests at least that a factor in fertilization is the rupture of a Graafian vesicle, due to orgastic reaction in the uterus, tubes and ovaries when copulation takes place immediately after menstruation. At this time ovulation is most likely to occur in lower animals, and all the facts indicate that the same is true in human beings. It is known that in the rabbit, dog and pig there must be repeated copulation in order to insure impregnation. A single mating rarely suffices. Thus, for instance, according to Weysse,[14] only three out of the nine sows became pregnant after being covered but a single time. This would indicate that the fertilization power of the sperm was of short duration, as Bryce and Teacher seem to think is the case in human beings.

According to Waldeyer[15] live spermatozoa were found in the bitch eight days after copulation, and dead cells, that is motionless cells at the end of 17 days. Living moving spermatozoa were found in a woman three days after death. Living sperm cells were found in the Fallopian tube of a patient 9 days after admission to the hospital and 3½ weeks after copulation. On the other hand, spermatozoa have been found upon the surface of the ovary of the rabbit and sow two hours after copulation. In Waldeyer's opinion the power to fertilize remains as long as the sperm cells retain normal motility, and there are no facts to deny that human sperm has the power to fertilize over a week after copulation.

Spermatic cells of animals that emit them into water die in a very short time if they are greatly diluted, and have a much longer life if only a little water is added. Thus in fertilizing trout eggs 'dry' sperm is used, while if the sperm is added to water containing the eggs but few eggs are fertilized. This

[14] Weysse, Arthur Wisswald 1894 The blastodermic vesicle sus scrofa domesticus. Proc. Amer. Acad. Arts and Sciences, vol. 30.

[15] Waldeyer, W. 1906 Hertwig's Handbuch der Vergleich. und Exper. Entwickelungslehre der Wirbeltiere. Bd. 1, Tl. 1, Erste Halfte.

question has been tested recently in Arbacia by F. R. Lillie,[16] who makes the following interesting statements: The spermatozoa are absolutely immobile while they are in the body of the male, but become intensely active when suspended in sea-water. They then become relatively inactive, but can be restored again by the addition of fresh sea-water. When greatly diluted they lose their fertilizing power completely in about an hour, and when diluted by 250,000 times their volume in water this power lasts but a few minutes. The loss of fertilizing power cannot be due to a loss of motility, for long after the former occurs no loss of vitality or motion is observed. In man the secretion of the prostate gland maintains the motility of spermatozoa much more effectively than does normal saline solution, and it is said that the secretions of the mucous membrane of the uterus and tubes have a similar influence. Thus it would seem that when motility is accelerated it does not indicate that the power to fertilize is prolonged, as asserted by Waldeyer. Lillie's experiments certainly do not favor such a view, and Bryce and Teacher infer the same when they state that were the spermatozoa to retain for a long time their power of fertilization, no ovum could escape fertilization.

For the sake of argument Bryce and Teacher deduct 24 hours from the copulation age of their specimen ($16\frac{1}{2}$ days) and estimate that it would have been $15\frac{1}{2}$ days old had it lived up to the time of abortion. This seems to me to be reasonable, as are the other statements in their admirable paper.

In view of the difference between the fertilization power of spermatozoa and their motility, as expressed in Lillie's report, we may admit with considerable safety that the fertilization power of sperm is of shorter duration than is the power on the part of the egg to be fertilized. Furthermore, the theory that a fruitful copulation should be accompanied by ovulation at about the same time is a necessary one, in order to account for all of the combinations which are encountered in human beings. Nor is the assumption of Bryce and Teacher of an oestrus following

[16] Lillie, F. R. 1915 Analysis of variation in the fertilizing power of sperm suspensions of Arbacia. Biol. Bull., vol. 28.

menstruation untenable, and the possibility of a relation between orgastic reaction and ovulation is not to be overlooked.

An interesting study in this connection has been made by Siegel,[17] using the wives of German soldiers as his subjects. These women, who became pregnant during their husband's furlough, came to the maternity hospital to be confined, and

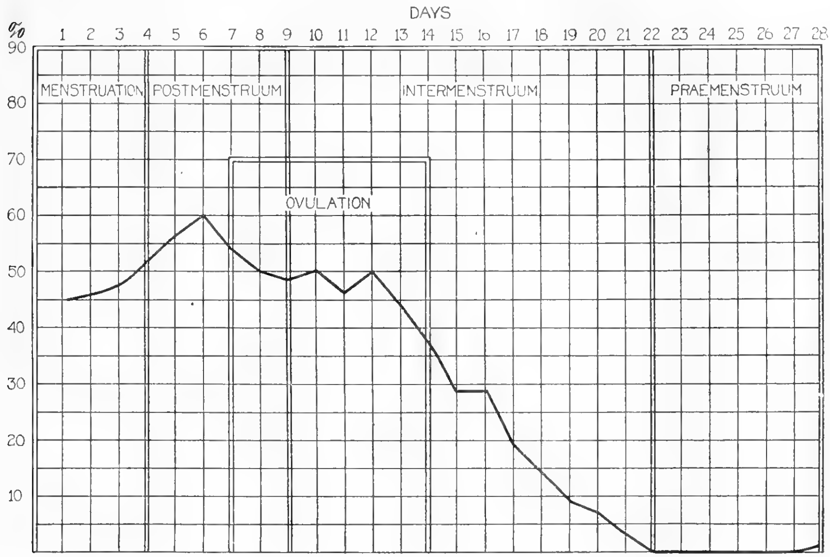

Fig. 2 Cohabitation curve according to Siegel. The main division of the menstrual month and the probable time of ovulation are given. One hundred cases of pregnancy, occurring in the wives of soldiers after their husbands' furlough of one week. Each day of the furlough is entered as a possible day of conception. In all probability the 1st to the 4th day and the 18th to the 21st day belong to the sterile portion of the month.

it was easy to obtain records of the menstrual history as well as the times of furlough, which in each case was of about a week's duration. Figure 2, taken from Siegel's paper, gives the result of the tabulation of 100 of such cases. Each day of the furlough is entered in the curve. Thus, if the furlough lasted from the

[17] Siegel, P. W. 1915 Warum ist der Beischlaf befruchtend? Deut. Med. Woch., 41.

8th to 16th day of the menstrual cycle it was entered for each of these days.

It is noteworthy that there was no entry for the last seven days of the menstrual month, indicating that pregnancy did not take place either a week before this nor within the week following; that is, there is a sterile period of about 18 days and a fertile period from the end of menstruation to the 15th day, which includes the probable time of ovulation. Of course only those cases which came to the maternity hospital could be recorded, and it is interesting to note that none of the 100 pregnancies dated from a furlough during the last week of the menstrual month. Such did not end in conception. Siegel was able to gather 10 cases in which the husband was on furlough a few days before the menstrual period, and in none did pregnancy follow. He cites further cases gathered by Wohler from the records of the same maternity hospital for the past ten years. These included 160 pregnancies among newly married women, in whom conception had occurred during the first five weeks after marriage. Among this group there were 65 cases in which marriage took place within the eight days preceding the menstrual period, and in each of them one more menstruation followed, which fact alone would indicate a sterile condition during the week preceding it. The records of Siegel, although not entirely satisfactory, demonstrate quite conclusively that the most probable time for conception is during the week or ten days after the period of menstruation.

From what has been written above we may, for the sake of argument, accept one day as the average time between copulation and fertilization. The time at which this is most likely to occur is between the 4th and 13th day after the first day of menstruation, as shown by the following table. This table is compiled from the records of our own cases, given above, each datum being obtained by subtracting the copulation age from the menstruation age. A similar result is obtained by dividing the total number of days by the number of cases, which equals exactly 13. That is, the average copulation date is the 13th day after the beginning of the last menstrual period. The figures upon which this result are

based are not altogether satisfactory, but if the six cases given in the table are recorded, the average date is one day later; that is, the 14th. The figures of these cases are as follows:

TABLE 6

Days between menstruation and copulation	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
No. of cases	1	0	0	1	2	2	1	3	1	4	2	3	1	0	1

Days between menstruation and copulation	16	17	18	19	20	21	22	23	24	25	26	27	28	30
No. of cases	0	1	1	3	2	1	2	0	1	0	0	1	1	1

TABLE 7

AUTHOR	LENGTH OF EMBRYO	TIME BETWEEN FIRST DAY OF MENSTRUATION AND COPULATION
	mm.	*days*
Bryce and Teacher	0.15	22
Eternod	1.3	13
Tandler	8.8	4
Rabl	14.0	18
No. 1390	18.0	7
No. 26	25.0	19

In this group just half of the cases date from the first two weeks of the menstrual cycle, and the other half from the second two weeks. It is recalled that in over 1000 cases of full term births the mean as computed from the time of menstruation and of copulation duration of pregnancy differed exactly 11 days, and that in my curve a difference of 10 days was taken. The theory of Bryce and Teacher and of Triepel is that fertilization takes place two days after copulation, and, therefore, they figure two days less than I did as the mean ovulation or fertilization age. Between these two theories I presume that we are within two or three days of the real age.

It appears to me probable that fertilization takes place nearly always within the uterine tubes—very rarely upon the surface of the ovary, as ovarian pregnancy is extremely rare; and probably also because the spermatozoa have lost their fertilizing power by the time they have passed the tube. No doubt this power is greatest in the tubes, as in these narrow channels the sperm would

not be unduly diluted, but instead there would be a tendency to bring it together again. The ovum would probably be fertilizable for fully 24 hours after ovulation, this time being sufficient to bring it into the outer end of the tube. The following table gives the copulation age of the rat's ovum, according to Huber, and of the dog's, according to Bischoff. I have taken the measurements from their illustrations which are to scale. The third column gives the greatest diameter of the human ovum, with the length of the embryo in the 4th column. The second portion of the table gives the fertilization age for the third week, according to Bryce and Teacher and to Triepel. It will be seen that the former allows 48 hours for fertilization after copulation, a period of time which, in my opinion, is abundantly long. Finally Triepel's column is practically identical with that of Bryce and Teacher.

TABLE 8

COPULATION AGE					FERTILIZATION AGE	
Age	Rat (Huber)	Dog (Bischoff)	Human Ovum	Embryo	Embryo (Bryce and Teacher)	Age (Triepel)
days	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*
1	0.07	0.15				
2	0.07	0.14				
3	0.09	0.14				
4	0.10	0.16				
5	0.11	0.16				
6	0.25	0.18				
7	0.40	0.20				
8	0.65	0.21				
9	1.30	0.28				
10		0.30				
11		1.00				
12		2.00				
13		3.00				
14		4.00			0.15	0.15
15		5.00			0.19	0.19
16		5.00	2.0	0.15		
17		6.00	3.5	0.2		0.37
18			5.0	0.3	0.37	
19			6.5	0.6	1.3	1.3
20			8.0	0.9	1.54	1.54
21			10.0	1.3		2.15

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DETERMINATION OF THE SIZE OF THE HEART BY MEANS OF THE X-RAYS

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ONE FIGURE

CONTENTS

The x-rays are of value in the study of the relations, the shape, the action and the size of the heart. We shall treat here of methods of determining the size of the heart and the relation of the size of the heart to the size of the body.

Of all the organs the heart is probably normally the most closely related in size to the size of the body as a whole. It is well known that a noticeably enlarged heart usually means some lesion either of the heart itself or of the blood vessels. Undersized hearts have been less studied but the more accurate methods of studying now being developed in x-ray technique show that it is of clinical importance to know when a heart is disproportionately small as well as when it is disproportionately large.

1. THE HEART SILHOUETTE

Of the various methods which have been devised for the study of the size of the heart those which have proved of greatest value are the orthodiagraphic and the teleroentgenographic. Ortho-

diagraphy has the advantage of giving a graphic outline which theoretically at least, corresponds exactly in size to the contour of the object casting the shadow and it is economical in material, but it takes much time and skill to exercise and is subject to errors when a moving organ like the heart is studied. Teleroentgenography with our modern machines is quick and accurate but demands that a proper allowance be made for enlargement of the heart silhouette due to divergence of rays.

Fortunately this is relatively simple when the distance from the target to the plate is the usual two meters and the patient faces the plate.

The average distance of the heart contour from the front of the chest is approximately one-third of the distance from the front to the back of the thorax measured at the lower part of the sternum during expiration. Albers-Schönberg ('08) has shown that the greatest transverse diameter of the heart lies in a plane parallel to the front of the thorax and at a distance of about one-third of the distance from the front to the back of the thorax at the level of the 6th thoracic vertebra. I have been able to confirm this observation by studies on numerous cadavers and on cross sections of the trunk and also to show that the average distance of the contour of the heart which casts the outline of the heart silhouette in parallel dorso-ventral rays is about the same distance from a plane parallel to the front of the chest. The contour of the apex is of course nearer the plate than the contours of the right atrium and the left atrium (W. Guttmann, '06) but we are concerned with the average distance of the heart contour from the plate. I have substantiated these studies on the cadaver by means of stereoscopic methods and half-distance methods of study of the distance of the heart contour from the plate in the living. Knowing the average distance of the heart contour from the plate it is possible to calculate the percentage of reduction which one must make of the heart silhouette in order to get the true size of the heart contour. In round numbers the silhouette area must be decreased one per cent for each three centimeters of distance from the front to back of the chest and a given diameter one per cent for each six centimeters. As a

routine for an adult of average size six per cent reduction of the silhouette area or three per cent reduction of a given diameter will give the actual size of the heart contour with sufficient accuracy to obviate the necessity of measuring the antero-posterior diameter of the chest and making a special calculation. But for very large or very small individuals and for children the simple formula given above should be followed.[1]

[1] When a shorter distance from the target to the plate than two meters is used, allowance must be made not only for this variation in distance but also for a variation in distance of the heart contour from the plate which enters in as the target is brought nearer to the heart. This factor also enters in at the two meter distance in ventro-dorsal pictures, the heart contour being further from the front of the chest in the dorso-ventral than in the ventro-dorsal position. For determining the average distance of the heart contour from the plate the stereoscopic method gives the best results. This is based on the distance of corresponding points in two silhouettes from a fixed line on the plate perpendicular to the line of shift of the tube. The average distance of a series of such points on the contour gives the average distance of the contour.

Knowing the distance of the target from the plate, the length of the shift of the tube and the distance of the shift of a given point in the two silhouettes it is easy to calculate the distance from the plate of the point on the heart which casts this point of shadow. The formula is $x = \frac{A.C}{A + B}$ where

x = distance of point on heart contour from the plate
A = distance of shift of given point in the two silhouettes
B = distance of shift of tube
C = distance from the target to the plate.

The formula for determining the relation of the size of the area of the silhouette to that of the area enclosed by the heart contour is as follows:

$$x = \frac{100\ B^2}{A^2}$$

x = area enclosed by the heart contour
100 = area of silhouette
A^2 = square of the distance from the target to the plate
B^2 = square of the distance from the target to the heart contour

The formula for determining the relation of a given diameter of the heart silhouette to a given diameter of the heart is as follows:

$$x = \frac{100\ B}{A}$$

x = length of diameter of heart contour
100 = length of diameter of silhouette
A = distance from the target to the plate
B = distance from the target to the heart contour

2. POSITION OF THE BODY IN RADIOGRAPHY OF THE HEART

In the orthodiagraphic studies the position of the patient is determined by the convenience of the operator and patient and general physiological considerations. It makes no particular difference whether the tube is behind or in front of the patient. In teleroentgenography it is important to have the heart as near the plate as possible and hence as a rule the patient should face the plate. It is difficult to place the tube two meters from the plate when the patient is in the supine position. The prone position is inconvenient and somewhat unnatural. A sitting or standing position as a rule is more convenient and comfortable. I have found the best position for routine work to be the sitting position in which the patient leans slightly forward against a plate holder with an inclination of 20° from the vertical. The ventral surface of the gladiolus of the sternum should be approximately parallel with the surface of the x-ray plate. This position

In the two-distances method of estimating the size of the heart two pictures are taken one with the target a shorter distance from the plate than the other. The most convenient distances are one and two meters. When these distances are chosen the following formulae may be used:

(1)

$$x = \frac{200\,A - 200\,a}{2\,A - a}$$

x = distance of plane of the heart contour from the plate
A = square root of area of heart silhouette in picture taken at one meter
a = square root of area of heart silhouette in picture taken at two meters.
If desired a given diameter may be used instead of the square root of the area.

(2)

$$x = \frac{A\,.\,a}{2\,A - a}$$

x = diameter of the heart
A = diameter of the heart silhouette at one meter
a = diameter of the heart silhouette at two meters

(3)

$$x^2 = \frac{a^2\,(200\text{-}x)^2}{200^2}$$

x^2 = area enclosed by heart contour
a^2 = area of silhouette at two meters
$200-x$ = distance from the target to the plane of the heart contour.

is comfortable and throws the heart forward toward the plate. The tube is raised high enough to direct the central rays perpendicular to the center of the plate. These central rays pass approximately through the tenth thoracie vertebra. As a routine the pictures are taken during deep but not forced inspiration and with two half second exposures with an intervening half second so as to insure a diastolic outline. For special studies we have taken pictures in the prone and standing positions as well as in the sitting position, during inspiration as well as during expiration. We have also taken instantaneous pictures timed by special electrical devices at any desired period of the cardiac and respiratory cycles.

The studies of Moritz, Dietlen ('09), and others have shown that as a rule the heart is larger in the supine than in the sitting position and in the sitting than in the standing position. According to Dietlen the difference as a rule is more marked in young healthy individuals than in those with less healthy hearts. For normal individuals he gives the average percentage difference in area of heart silhouette between the supine and standing positions as 20 per cent of the supine area with the extremes at 30.4 per cent and 10.6 per cent. For those with slight lesions he found an average difference of 12.8 per cent, those with marked lesions a difference of 9.5 per cent and in cases of recent decompensated hearts a difference of only 5.6 per cent. On the other hand in some cases of acute dilatation he found variations of from 23.3 per cent to 34.6 per cent.

Dietlen's figures for normal individuals seem somewhat high. (Cf. Otten, '11–'12.) In eight normal individuals, taking the pictures during deep inspiration, I found an average difference in area between the prone position and the sitting position of 6.7 per cent of the prone area (extremes 2.8 per cent and 12.1 per cent) and between the prone and standing positions of 13.3 per cent (extremes 2.4 per cent and 17.4 per cent). In nine other individuals of whom 'instantaneous' pictures were taken in the prone and sitting positions during quiet respiration at the height of diastole I found an average difference of 4.7 per cent of the prone area (extremes 0 per cent to 10.9 per cent). Veith's

studies of twenty-three boys in the supine and sitting positions ('08) show an average difference of 7.6 per cent between the supine and the sitting positions (extremes 2.6 per cent to 22.7 per cent). For the average normal individual we may therefore take 5 to 7 per cent as a conservative estimate of the reduction in area which we may expect in the heart shadow area between pictures taken in the prone and sitting positions when the patient leans slightly forward in the latter position. These changes in size of the heart associated with change in posture are due chiefly to changes in hydrostatic pressure in the inferior vena cava. To a large extent they may be overcome by binding the lower extremities. As a rule but not always the pulse rate is faster in postures in which the heart is relatively small.

The effects of the respiration on the size of the heart seem to be less constant. According to F. M. Groedel ('11) the heart does not as a rule change in size during quiet respiration although there is a slight fall of blood pressure during inspiration. In forced inspiration there is a marked fall of blood pressure which may be followed by a passive rising of the diaphragm and a rise of blood pressure. At the height of deep inspiration the heart, or at least the heart shadow, may be smaller than normal owing to the pull of the pericardium. In three experiments in the sitting position I found a decrease in the area of the heart silhouette in expiration as compared with inspiration in two instances (−2 per cent, −2.5 per cent), an increase in one instance (+5 per cent), average +0.2 per cent. In the prone position I found a decrease in the cardiac area during expiration in two instances (−5 per cent, +6 per cent) and an increase in one instance, +4 per cent), average −2.3 per cent. In the standing position I found an increase during expiration of 6.6 per cent and 9 per cent in two instances, average +7.8 per cent. From these few experiments it would appear that in the prone position, when the heart is relatively large in size, it tends to be smaller during expiration, while in the standing position, in which the heart is relatively small, the heart tends to be larger during expiration than during deep inspiration. We need a much more extended series of observations before this can be

considered definitely determined. In the position we have chosen as a standard it is probable that a moderately deep inspiration increases the diastolic filling to a slight extent over that in expiration. The negative pressure produced in the thorax by the inspiration tends to fill the heart during diastole, while no marked restraint is exercised by the pericardium.

3. MEASUREMENT OF THE HEART SILHOUETTE

Methods of measuring the heart silhouette vary. As a rule the right and left margins of the heart silhouette are clearly defined while above the heart silhouette merges with that of the great vessels and vertibral column and below with that of the diaphragm, liver and stomach. The apex of the heart is most clearly defined when there is a well marked gas accumulation in the stomach. Sometimes a Seidlitz powder is given a patient in order to insure a well marked gas bubble in the stomach. The pressure of an excessive amount of gas may, however, to some extent distort the picture. As a rule if the patient is given a glass or two of water immediately before the picture is taken and is requested to swallow as much air as possible with the water a gas bubble of sufficient size will be formed in the stomach to aid in outlining the heart. But gas in the stomach does not serve to make a clear demarcation between the shadow of the heart and that of the liver. To complete the lower margin of the heart shadow it is necessary to draw a line to connect the outline of the left margin with that of the right margin of the heart.

With practice it becomes possible to draw this line with fair approximation to its true position. As one gets used to visualizing the heart one learns to continue the swing of the line from the right side into that from the left side. I have drawn many hearts in position in the dissecting room using an apparatus which enables me to draw a line perpendicularly above the margin of the heart. By comparing these drawings with those made from x-ray plates it becomes evident that the approximately correct completion of the lower margin of the heart outline is less difficult than one might expect. It is more difficult in fat than in thin individuals.

At the base of the heart a purely arbitrary line must be drawn since there is no simple line of demarcation between the heart and the great vessels. If, however, the right and left margins of the heart silhouette be connected by a line which curves gracefully from the curve of the right margin into that of the left we have a line which will include within the territory of the heart the right and left atria and the cardiac extremity of the pulmonary artery and of the aorta. A small portion of the left auricle may be cut off by the line that curves toward the right from the left border but as a rule this is insignificant (fig. 1).

By practice in employing this method of outlining the heart silhouette, which is essentially that of Mortiz and Dietlen, one may acquire sufficient skill to make practically identical estimates of the heart silhouette area when plates are studied at widely different intervals. This is perhaps the best test of one's own consistency with the method. Different observers may establish slightly different methods of completing the outline of the heart silhouette which will lead to slightly different estimates of the heart silhouette area but these differences should not be serious when careful studies are made of the anatomy of the heart in the dead body in conjunction with the heart shadow in the living. The extent of the area included within the outline of the heart shadow may be quickly estimated with a planimeter. If the outline is that of a teleroentgenograph the appropriate reduction for ray divergence should then be made.

The chief objection to the method of estimating the size of the heart from the heart silhouette area as outlined above is that it is not sufficiently objective. For this reason it has not been used by a number of foreign and American investigators who have made x-ray studies of the heart. Among these may be mentioned Otten ('12), Groedel ('08), Claytor and Merrill ('09), Williamson ('15) and Shattuck ('16).

The most objective measurement that can be made of the heart silhouette is that of the greatest transverse diameter. This is probably the measurement most frequently made. For the study of comparative size the transverse diameter of the heart shadow is compared with the transverse diameter of the

thorax according to some such formula as that suggested by Kreuzfuchs ('12).

Some investigators add to the measurement of the transverse diameter of the heart the measurement of the long diameter from the point where the curve of the right border of the heart is broken by the line of the aorta or of the superior vena cava to the apex of the heart silhouette. Since, however, an accurate outline of the apex of the heart is the chief difficulty that confronts one when he attempts to complete the line of the lower border of the heart the measurement of the long diameter of the heart is subject to the chief error that may arise from measuring the area of the silhouette and the long diameter gives a far less satisfactory standard on which to base an estimate of volume. This is true of the numerous other diameters that may be measured on the heart silhouette. The area, which combines them all, gives the best standard from which to estimate the volume of the heart. For study of variations in the shape of the heart however, some of these various diameters may be of value.

4. TABLES A AND B

Chosing then the area of the heart silhouette, reduced in case of radiographs to conform in size to the contour of the heart, (see p. 424) as the standard from which to estimate the size of the heart we have tabulated in tables A and B the normal relations of a silhouette area of a given size to transverse diameter, to body weight, to heart weight, to heart volume and to height in either sex at various ages. The data on which these estimates are based may be summarized as follows:

a. Heart silhouette area and body weight

The relations of silhouette area to body weight are based primarily on the study of radiographs of 188 men, 42 women and 9 children, all healthy and normal from the clinical standpoint which we have studied at the Wisconsin Clinic according to the teleroentgenographic method outlined above. With the data obtained from these studies have been compared the orthodia-

TABLE A

Table showing relations of a heart silhouette area of a given size to approximate transverse diameter, body weight, heart weight, heart volume in diastole, and height for either sex, at a given age during childhood. Individual at rest, sitting

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	SEX	AGE YEARS	ESTIMATED HEIGHT	
sq. cm.	*cm.*	*kilos*	*pounds*	*grams*	*cc.*			*cm.*	*inches*
16	4.7	3.2	7.1	17.5	34			51	20
17	4.9	3.5	7.7	19.0	37		BIRTH	52	
18	5.0	3.8	8.4	21.0	40			53	21
19	5.1	4.1	9.1	23.0	44		$\frac{1}{12}$	54	
20	5.3	4.5	9.8	25.0	47			56	22
21	5.4	4.8	10.6	26.5	51			57	
22	5.5	5.2	11.4	28.5	55		$\frac{1}{6}$	58	23
23	5.7	5.5	12.1	30.5	59			60	
24	5.8	5.9	12.9	32.5	62		$\frac{1}{4}$	61	24
25	5.9	6.3	13.8	34.5	66			62	
26	6.0	6.6	14.6	36.5	70		$\frac{1}{3}$	64	25
27	6.1	7.0	15.5	28.5	74			65	
28	6.2	7.4	16.4	41.0	79		$\frac{1}{2}$	66	26
29	6.4	7.8	17.2	43.0	83			67	
30	6.5	8.2	18.1	45.0	87		$\frac{3}{4}$	69	27
31	6.6	8.6	19.0	47.0	92			70	28
32	6.7	9.0	20.0	49.5	96			72	
33	6.8	9.5	21.0	52.0	101		1	74	29
34	6.9	9.9	22.0	54.5	105			76	30
35	7.0	10.4	23.0	57.0	110			78	
36	7.1	10.8	24.0	59.5	115			80	31
37	7.2	11.2	25.0	62.0	119		2	81	32
38	7.3	11.7	26.0	64.5	124			83	33
39	7.4	12.2	27.0	67.0	129			85	
40	7.5	12.6	28.0	69.5	134			87	34
41	7.6	13.0	29.0	72.0	139		3	89	35
42		13.5	30.0	74.5	144			91	36
43	7.7	14.0	31.0	77.0	149			93	
44	7.8	14.5	32.0	80.0	155			95	37
45	7.9	15.0	33.0	82.5	160		4	97	38
46	8.0	15.5	34.0	85.0	165			99	39
47	8.1	16.0	36.0	88.0	171			101	40
48	8.2	16.5	37.0	91.0	176	F	5	103	41
49	8.3	17.0	38.0	94.0	182	M	5	104	41
50		17.5	39.0	97.0	187			106	42
51	8.4	18.0	40.0	100.0	193			108	43
52	8.5	18.5	41.0	103.0	198	F	6	110	

TABLE A—Continued

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	SEX	AGE YEARS BIRTH	ESTIMATED HEIGHT	
sq. cm.	*cm.*	*kilos*	*pounds*	*grams*	*cc.*			*cm.*	*inches*
53	8.6	19.0	43.0	106.0	204	M	6	112	44
54	8.7	20.0	44.0	109.0	210			114	45
55	8.8	20.5	45.0	1[illegible]	216	F	7	116	
56		21.0	46.0	115.0	222	M	7	117	46
57	8.9	21.5	47.0	118.0	228			119	47
58	9.0	22.0	49.0	122.0	234	F	8	121	
59	9.1	22.5	50.0	125.0	240	M	8	122	48
60		23.0	51.0	128.0	246			124	49
61	9.2	24.0	53.0	132.0	253	F	9	126	
62	9.3	24.5	54.0	135.0	259	M	9	127	50
63		25.0	55.0	138.0	265			129	
64	9.4	25.5	56.0	141.0	271			130	51
65	9.5	26.0	58.0	144.0	278	F	10	131	
66	9.6	27.0	59.0	147.0	284	M	10	132	52
67		27.5	61.0	150.0	290			133	
68	9.7	28.0	62.0	154.0	297			134	53
69	9.8	28.5	63.0	158.0	304	F	11	135	
70		29.5	65.0	161.0	311	M	11	136	54
71	9.9	30.0	66.0	165.0	317			137	
72	10.0	30.5	67.0	168.0	324			138	
73	10.1	31.0	69.0	172.0	331			139	55
74		32.0	70.0	175.0	337			140	
75	10.2	32.5	72.0	179.0	344	F	12	141	56
76	10.3	33.0	73.0	182.0	351	M	12	142	56
77		34.0	75.0	186.0	358			143	
78	10.4	34.5	76.0	189.0	365			144	
79	10.5	35.0	77.0	193.0	372	M	12½	145	57
80		36.0	79.0	197.0	379			146	
81	10.6	36.5	80.0	201.0	386			147	
82	10.7	37.0	82.0	204.0	393	M	13	148	58
83		38.0	83.0	208.0	401			149	
84	10.8	38.5	85.0	212.0	408	F	13	150	59
85		39.0	86.0	215.0	415			151	
86	10.9	40.0	88.0	219.0	423			152	
87	11.0	40.5	89.0	223.0	430	M	14	153	60
88		41.5	91.0	227.0	438			154	
89	11.1	42.0	93.0	231.0	445			155	
90	11.2	42.5	94.0	235.0	453				
91		43.5	96.0	239.0	460	F	14	156	61
92	11.3	44.0	97.0	243.0	468				
93		45.0	99.0	247.0	475			157	

TABLE A—Concluded

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	SEX	AGE YEARS BIRTH	ESTIMATED HEIGHT	
sq. cm.	*cm.*	*kilos*	*pounds*	*grams*	*cc.*			*cm.*	*inches*
94	11.4	45.5	100.0	251.0	483				
95	11.5	46.5	102.0	255.0	490			158	
96		47.0	104.0	259.0	498	F	15	159	62
97	11.6	48.0	105.0	263.0	506	M	15	160	63
98	11.7	48.5	107.0	267.0	514				
99		49.5	109.0	271.0	522			161	
100	11.8	50.0	110.0	275.0	530	M	15½	162	64
101		51.0	112.0	279.0	538	F	16	160	63
102	11.9	51.5	114.0	283.0	546			163	
103		52.5	115.0	287.0	554			164	
104	12.0	53.0	117.0	292.0	562	M	16	165	65
105	12.1	54.0	119.0	296.0	570			166	
106		54.5	120.0	300.0	578	F	17	163	64
107	12.2	55.5	122.0	304.0	586	M	16½	167	66
108	12.3	56.0	124.0	309.0	595			168	
109		57.0	125.0	313.0	603			169	
110	12.4	58.0	127.0	317.0	612	M	17	170	67
111		58.5	129.0	322.0	620			171	
112	12.5	59.0	130.0	326.0	628				
113		60.0	132.0	330.0	637			172	
114	12.6	61.0	134.0	335.0	645				
115		61.5	136.0	339.0	653	M	18	173	68
116	12.7	62.5	138.0	343.0	662				

TABLE B

Table showing relations of a heart silhouette area of a given size to approximate transverse diameter, body weight, heart weight, heart volume in diastole, and height for either sex, at a given age. Individual at rest, sitting

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	HEIGHT AT GIVEN AGE FOR GIVEN WEIGHT								
						20 years			30 years			50 years		
						Sex	Height		Sex	Height		Sex	Height	
sq. cm.	*cm.*	*kilos*	*pounds*	*grams*	*cc.*		*cm.*	*in.*		*cm.*	*in.*		*cm.*	*in.*
93		45.0	99	247	475									
94	11.4	45.5	100	251	483	F	140	55						
95	11.5	46.5	102	255	490	F	142	56	F	130	41			
96		47.0	104	259	498	M	145	57	F	135	53			
97	11.6	48.0	105	263	506	F	145	57	F	137	54			
98	11.7	48.5	107	267	514	M	147	58	M	137	54	F	124	49.0
99		49.5	109	271	522	F	150	59	F	142	56	F	132	52.0
100	11.8	50.0	110	275	530	M	150	59	M	152	56	M	135	53.0
101		51.0	112	279	538	F	152	60	M	145	57	M	137	54.0
102	11.9	51.5	114	283	546	M	152	60	M	147	58	F	140	55.0
103		52.5	115	287	554	F	155	61	F	150	59	M	142	56.0
104	12.0	53.0	117	292	562	M	155	61	M	150	59	M	145	57.0
105	12.1	54.0	119	296	570	F	157	62	F	152	60	F	145	57.0
106		54.5	120	300	578	M	157	62	M	152	60	M	147	58.0
107	12.2	55.5	122	314	586	F	160	63	F	155	61	F	147	58.0
108	12.3	56.0	124	309	595	M	160	63	M	155	61	M	150	59.0
109		57.0	125	313	603	F	163	64	F	157	62	F	150	59.0
110	12.4	58.0	127	317	612	M	163	64	M	157	62	M	152	60.0
111		58.5	129	322	620				M	160	63	M	155	61.0
112	12.5	59.0	130	326	628	F	168	66	F	163	64	F	155	61.0
113		60.0	132	330	637	M	165	65	M	163	64	M	157	62.0
114	12.6	61.0	134	335	645	M	168	66	F	165	65	F	157	62.0
115		61.5	136	339	653	F	170	67						
116	12.7	62.5	138	343	662	M	170	67	M	165	65	M	160	63.0
117	12.8	63.5	140	348	671							F	160	63.0
118		64.0	141	352	679	F	173	69	M	168	66	M	163	64.0
119	12.9	65.0	143	357	688	M	173	68	F	170	67	F	163	64.0
120		65.5	145	361	696	F	178	70	F	173	68			
121	13.0	66.5	147	366	705	M	175	69	M	170	67	M	165	65.0
122		67.5	149	371	714	F	180	71	F	175	69			
123	13.1	68.0	150	375	723	M	178	70	M	173	68	M	168	66.0
124		69.0	152	380	732							F	168	66.0
125	13.2	70.0	154	384	741	F	183	72	F	178	70	F	170	67.0
126		71.0	156	389	750	M	180	71	M	175	69	M	170	67.0
127	13.3	71.5	158	394	759				F	180	71	F	171	67.5
128		72.5	160	398	768	M	183	72	M	178	70	M	173	68.0
129	13.4	73.5	162	403	777									

TABLE B—Continued

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	HEIGHT AT GIVEN AGE FOR GIVEN WEIGHT								
						20 years			30 years			50 years		
						Sex	Height		Sex	Height		Sex	Height	
sq. cm.	cm.	kilos	pounds	grams	cc.		cm.	in.		cm.	in.		cm.	in.
130		74.0	163	408	786				F	185	72	F	174	68.5
131	13.5	75.0	165	413	695	M	185	73	M	180	71	M	175	69.0
132		76.0	167	417	804									
133	13.6	76.5	169	422	813	M	188	74				F	178	70.0
134		77.5	171	427	823				M	183	72	M	178	70.0
135	13.7	78.5	173	431	832									
136		79.5	175	436	841	M	191	75	M	185	73	F	180	71.0
137	13.8	80.0	177	441	850							M	180	71.0
138		81.0	179	446	860	M	193	76				F	183	72.0
139	13.9	82.0	181	451	869				M	188	74	M	183	72.0
140		83.0	183	456	878									
141	14.0	83.5	185	460	887									
142		84.5	187	465	897				M	190	75	M	185	73.0
143	14.1	85.5	189	470	906				M	190	75	M	185	73.0
144		86.5	191	475	916									
145	14.2	87.5	193	480	925				M	193	76	M	188	74.0
146		88.0	195	485	934									
147	14.3	89.0	197	490	944									
148		90.0	199	495	954									
149	14.4	91.0	201	500	964							M	191	75.0
150		92.0	203	505	974									
151	14.5	93.0	205	510	984									
152		93.5	207	515	994							M	193	76.0
153	14.6	94.5	209	521	1004									
154		95.5	211	526	1013									
155	14.7	96.5	213	531	1023									
156		97.5	215	536	1033									
157	14.8	98.5	217	541	1043									
158		99.5	219	546	1053									
159	14.9	100.0	221	551	1063									
160		101.0	223	557	1073									
161	15.0	102.0	225	562	1083									
162		103.0	227	567	1093									
163		104.0	230	572	1104									
164	15.1	105.0	232	578	1114									
165		106.0	234	583	1124									
166	15.2	107.0	236	588	1134									
167		108.0	238	593	1144									
168		109.0	240	599	1154									

TABLE B—Concluded

HEART SILHOUETTE AREA	APPROXIMATE TRANSVERSE DIAMETER	WEIGHT OF BODY		WEIGHT HEART EMPTY	VOLUME HEART DIASTOLE	HEIGHT AT GIVEN AGE FOR GIVEN WEIGHT								
						20 years			30 years			50 years		
						Sex	Height		Sex	Height		Sex	Height	
sq. cm.	cm.	kilos	pounds	grams	cc.		cm.	in.		cm.	in.		cm.	in.
169	15.3	110.0	242	604	1164									
170		111.0	244	610	1175									
171	15.4	112.0	246	615	1185									
172		113.0	248	620	1195									
173	15.5	114.0	250	626	1206									
174		115.0	253	631	1216									
175	15.6	116.0	255	637	1227									
176		117.0	257	642	1233									
177	15.7	118.0	260	648	1249									
178		119.0	262	653	1259									
179	15.8	120.0	264	658	1269									
180		121.0	266	664	1280									
181		122.0	268	669	1290									
182	15.9	123.0	271	675	1301									
183		124.0	273	681	1312									
184	16.0	125.0	275	686	1322									
185		126.0	277	692	1333									
186	16.1	127.0	279	697	1344									
187		128.0	282	703	1355									
188	16.2	129.0	284	708	1366									
189		130.0	286	714	1377									
190	16.3	131.0	288	720	1388									
191		132.0	290	726	1399									
192		133.0	293	732	1410									
193	16.4	134.0	295	737	1421									
194		135.0	297	743	1432									
195		136.0	299	748	1443									
196	16.5	137.0	301	754	1454									
197		138.0	304	759	1465									
198	16.6	139.0	306	765	1476									
199		140.0	308	770	1487									
200		141.0	310	776	1499									

graphic data of Dietlen ('07) on 187 men and 74 women, of Schieffer ('07), on 123 men, of Veith ('08), on 80 orphan boys in the prone position, 25 orphan boys in the sitting position and 25 school boys and 25 school girls in the sitting position. I have also compared with these data data from Claytor and Merrill ('09), based on the formula suggested by these authors that the area of the heart shadow equal 70 per cent of the product of the long diameter of the heart shadow by the transverse diameter. These data relate to 37 men and 54 women. For the purpose of comparison I have included similar estimates based on 156 of the men studied by Dietlen and on 100 men studied by Otten ('12). With these x-ray studies we have combined a direct study of the heart contour in the dissecting room by means of drawing on a glass plate so that the outline of the drawing is perpendicular to the contour of the heart.[2]

From this study of the relation of the size of the heart silhouette to the body weight the following formula was determined:

$$B = \frac{1}{20} H^{3/2}$$

where

B = weight of body in kilograms.

H = area of heart silhouette in square centimeters. (Bardeen '16).

The values for body weight corresponding to a given area of heart silhouette in tables A and B are based upon this formula. For the sake of simplicity the weight is given in round numbers in kilos and half kilos for body weights above 12.6 K.; in even pounds for 19 pounds and above.

The following tables show the average percentage of divergence of the different groups of cases studied from the standard.

The average percentage of divergence is determined as follows: The heart silhouette area of each individual in the group is compared with the area estimated to correspond with the body weight

[2] This is accomplished by means of an arc lamp, lenses and a mirror which throws parallel rays of light from above through a glass plate so that the shadow of a pencil point may be followed about the margin of the heart as the outline is sketched on the glass plate.

of the individual. The difference between the observed and the estimated areas is divided by the estimated area and the resulting quotient is the per cent of divergence for that individual. Thus if the individual weighs 50 K. (110 lbs.) one would estimate a heart silhouette area of 100 sq. cm. If the observed silhouette area is 110 sq. cm., it is 10 sq. cm. above the estimated or +10 per cent. If the observed silhouette area is 90 sq. cm. it is 10 sq. cm. below the estimated or −10 per cent. The average of these divergences for the group gives the average percentage of divergence

For normal children below four years of age we have at present no data as to heart silhouette area in relation to body weight. Table A has been completed down to birth in order to give a working basis for study of the size of the heart in these younger children. The statistical data given by various investigators as to heart weight in relation to body weight in young children make it probable that the formula of the relation of heart silhouette area to body weight holds approximately true of young children as well as of older individuals.

For children from 15 to 40 K. in weight (33 to 88 lbs.) the most extensive data are those of Veith (table 1). He gives figures showing the heart silhouette area of 80 boys (orphans) in the prone position and of fifty boys and twenty-five girls taken sitting by the orthodiascopic method. The general average of the eighty boys in the prone position is 10.8 per cent above expectation. Of twenty-five orphan boys sitting, twenty-three of whom were also in the first group, the general average is 2.1 per cent above exceptation. We may therefore attribute the major part of the excess size of the hearts of the boys of the first group to the prone position in contrast to the sitting position on which our standard table is based. Three of the boys in the 31–40 K. group and six in the 21–30 K. group have very large heart silhouettes. If these be excluded the excess of size of the average shadow area about equals what would be expected from the prone position. Of the other groups the twenty-five school boys sitting show an average divergence of only −0.08 per cent while the school girls sitting show an average divergence of −5.1 per cent. It is probable that the formula for the relation

of the heart shadow area to body weight gives figures for the heart shadow area slightly too high for the average girl or woman, as we shall point out below in discussing adults. The few young girls I have studied, however, show heart shadow areas corresponding perfectly to the standard while the boys show an average excess of 1.1 per cent.

TABLE 1

Average percentage of divergence from the standard silhouette area corresponding to a given body weight in children

WEIGHT	OBSERVER											
	Veith						Bardeen					
	Boys, supine		Orphans sitting		Sitting school boys		Girls sitting		Boys sitting		Girls sitting	
	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence
15–20 K........ 33–44 lbs.......	8	+ 9.5	3	+3.0	1	+5.8	4	−6.7	4	+0.3	3	00
21–30 K........ 45–66 lbs.......	53	+12.5	13	+4.6	15	−0.8	8	−0.1	1	−2.3		
31–40 K........ 67–88 lbs.......	19	+ 9.3	19	−2.1	7	−1.1	12	−4.2	1	+7.5		
41–50 K........ 89–110 lbs......					2	−2.1	1	−7.8				
Totals.........	80	+10.8	25	+2.1	25	−0.8	25	−5.1	6	+1.1	3	00

The average divergence from the standard of the 188 normal men studied by me is −0.1 per cent (table 2). The most noteworthy feature of the divergencies of the sub-groups is that shown by the heavier sub-groups, a decreasing size of the heart silhouette relative to the body weight. This is also shown in the athlete column, the lighter sub-groups with one exception showing relatively large silhouette areas while the heavier sub-groups show smaller relative areas. Among the athletes here tabulated are included men who have taken an active part in strenuous intercollegiate athletics but we have excluded from the table athletes whose hearts gave clinical evidence of abnormality. The forty-

two women with clinically normal hearts studied at the Wisconsin Clinic show hearts relatively slightly small for the lighter groups, more markedly small for the heavier groups. While it appears that the standard of heart silhouette area in relation to

TABLE 2

Average percentage of divergence from the standard silhouette area corresponding to a given body weight in youths and adults

WEIGHT	OBSERVER											
	Bardeen, sitting position						Dietlen, supine position				Schieffer supine position	
	Men		Athletes		Women		Men		Women		Men	
	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence
40–50 K.... 88–111 lbs. .	1	− 3.0	2	+21.9	9	− 1.0	17	+1.8	20	− 1.6		
51–60 K... 112–133 lbs..	25	+ 2.2	4	− 4.6	23	− 1.6	82	+1.9	38	− 8.1	55	+8.6
61–70 K... 134–155 lbs..	90	+ 1.4	14	+ 4.4	9	− 3.4	72	−1.6	16	−10.5	56	+3.2
71–80 K... 156–178 lbs..	47	− 1.4	8	+ 2.7	1	−28.4	12	−0.3			.10	+2.8
81–90 K... 179–200 lbs..	19	− 4.7	2	− 2.9			4	−9.5			2	−1.1
91–100 K... 201–221 lbs..	4	− 5.0										
100 K....... 222 lbs......	2	−13.0										
Totals	188	− 0.1	30	+ 3.4	42	− 2.5	187	−0.01	74	− 6.6	123	+5.5

body weight given in tables A and B is slightly large for the average women the difference between the size of the silhouette for men and women of a given weight is so little, probably not over 2.5 per cent for individuals of average size, that it does not seem worth while to try to establish a separate curve for women.

The relatively smaller heart silhouettes of fat individuals appears in groups of lighter weight for women than for men because heavy women average less in height. See below for a discussion of the effects of height.

The figures in table 2 based on Dietlen's data show on the average a close correspondence for the 187 men, a divergence of only -0.01 per cent. We should, however, expect in this group an average plus divergence of over 5 per cent since Dietlen's observations were made on individuals in the supine position while the standard table is based on individuals in the sitting position. The relatively small size of the silhouette area in Dietlen's figures may be due in part to differences in method and in part to racial differences.

The relatively small size of the heart silhouette in Dietlen's studies of women is more marked than in those studied by me. Schieffer's studies of the hearts of individuals engaged in strenuous muscular work show an average increase of 5.5 per cent of the size of the heart shadow above the normal but since his studies were made on individuals in the prone position, we should expect about this difference from a standard based on the sitting position. The lighter groups of individuals show relatively large heart shadows.

Giegel ('14) suggested as a method of determining the heart quotient, the division of the 3/2 power of the area of the heart silhouette by the body weight in kilograms. He showed that the heart quotient thus obtained varied in Dietlen's cases from fifteen to twenty-three in 93 per cent of the cases. The extremes were twenty-seven (two cases) and fourteen (three cases). Expressed in terms of divergence from the normal standard this would mean $+21$ per cent to -9 per cent for the 93 per cent of cases, -18 per cent and $+27$ per cent for the extremes. The amount of divergence from the standard found in the normal individuals in the groups studied by me will be discussed below in connection with other factors which must be considered, age and height.

The area of the cardiac silhouette estimated as 70 per cent of the long diameter of the heart times the transverse diameter gives for Claytor and Merrill an average divergence of -5.7

per cent, for males and −15.3 per cent for females. The Dietlen males, on the other hand, show by this method of estimation an average divergence of +12 per cent. By direct measurement of the area enclosed by the completed cardiac outline, the average divergence in 187 men studied by Dietlen is −0.01 per cent. The average divergence of the cardiac shadow of the men studied by Otten is +4.9 per cent. Making allowance for the prone

TABLE 3

Average percentage of divergence from the standard silhouette area corresponding to a given body weight in youths and adults Area estimated as 70 per cent long diameter times transverse diameter.

WEIGHT	OBSERVER							
	Claytor and Merrill				Dietlen		Otten	
	Men		Women		Men		Men	
	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence
40–50 K. / 88–111 lbs					5	+ 7.5	23	+9.9
51–60 K. / 112–133 lbs	3	−12.0	19	−13.3	72	+16	36	+4.8
61–70 K. / 134–155 lbs	22	− 5.8	31	−15.9	64	+10.9	32	+2.6
71–80 K. / 156–178 lbs	6	− 3.0	4	−17.2	11	+12.2	9	−1.2
81–90 K. / 179–200 lbs	6	− 7.3			4	+ 9		
Totals	37	− 5.7	51	−15.3	156	+12	100	+4.9

position makes the average for the Otten figures correspond closely with the standard based on the sitting position. The heavier individuals show relative small cardiac shadow areas as in the case of the individuals tabulated in table 2.

In table 4 are given dissection room data obtained according to the method outlined above, p. 438. The bodies studied for this tabulation were all embalmed with equal parts of carbolic acid,

alcohol and glycerine. They were measured and weighed before being dissected and the heart was measured and weighed as soon as the thorax was opened. As a rule the embalming fluid was injected into the femoral veins under a pressure of six pounds. In many bodies this leaves the chambers of the heart moderately

TABLE 4

Average percentage of divergence from the standard silhouette area and standard heart weight corresponding to a given body weight from dissecting room data

AGE AND WEIGHT OF BODY	AREA HEART OUTLINE				HEART WEIGHT			
	Male		Female		Male		Female	
	Numbes	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence	Number	Per cent divergence
Foetus; 2.06 K., 4.5 lbs...	1	−23.0			1	−23.0		
New born; 2.9 K., 6.4 lbs...	1	−46.0			1	−37.3		
New born; 4.3 K., 9½ lbs...			1	+ 7.7			1	+20.5
Child 2½ years; 5.9 K., 13 lbs....................	1	+25.0			1	+ 1.7		
Child 4 years; 7.3 K., 16 lbs....................	1	− 5.8			1	−17.8		
Child 2 years; 8.6 K., 19 lbs....................	1	+ 9.7			1	+ 1.5		
Child 9 years; 20 K., 44 lbs....................	1	− 9.3			1	− 7.3		
Total, children.........	6	− 8.2	1	+ 7.7	6	−12.0	1	+20.5
Adults:								
21–30 K., 45–66 lbs......	1	+ 9.4	2	+ 9.7	1	− 8.3	2	+ 7.2
31–40 K., 67–87 lbs......	8	+ 3.0	6	+ 9.9	8	− 1.2	6	+ 0.17
41–50 K., 88–111 lbs......	10	+ 5.1			11	+ 0.7		
51–60 K., 112–133 lbs......	3	+ 0.5	1	−13.9	3	− 9.6	1	−25.2
61–70 K., 134–155 lbs......								
71–80 K., 156–178 lbs......	2	+ 3.2			2	− 6.1		
81–90 K., 157–198 lbs.								
Total, adults...........	24	+ 3.9	9	+ 7.2	25	− 2.0	9	− 1.1

distended. Such bodies were selected for this study. Bodies showing cardiac lesions or in which from one cause or another the heart was distorted were not utilized for the data here tabulated although frequently utilized for other data in the study of the heart. The weight of the heart was controlled and in some

instances estimated from the displacement of water or oil by the empty heart. While data of this kind are necessarily crude, especially since the bodies utilized were the regular material used in teaching medical students, it is believed the data obtained have some value, especially in connection with x-ray studies on the living.

Twenty-four adult male bodies show an average divergence from the normal standard of +3.9 per cent, nine females an average divergence of +7.2 per cent. The individual variations are to be attributed in part to variations in the extent of distention of the heart after embalming. The relatively few female bodies studied do not lend support to what seems to be on other evidence fairly well established that the female heart relative to body size is slightly smaller than the male.

The foetus and one of the new born infants studied show small hearts, the other new born, a large heart. One of the young children shows a small heart, the other two large hearts. The nine year old child shows a heart small from the standpoint of area and from the standpoint of weight. While the data here given for children are scanty they tend on the whole to lend support to the belief that the standards of tables A and B are approximately correct for young children.

b. Silhouette area and transverse diameter

The transverse diameter varies in size in relation to the area of the heart silhouette and the volume of the heart according to the position of the heart. If the long axis of the heart is transversely placed, as during childhood and in fat adult individuals, the transverse diameter of the silhouette is relatively large. If the long axis is more nearly vertical as is usual during youth and in thin adult individuals, the transverse diameter of the silhouette is relatively small. I have found far wider variations in the transverse diameter than in the area of the heart silhouette in relation to the size of the body. To compare the extent of variations of transverse diameter with those of area the former should be squared. If this be done the variations in transverse

diameter are from five to fifteen times as great. Dietlen's tables show the same thing.

We have therefore discarded the transverse diameter of the heart silhouette in favor of the area as a means of estimating relative heart size. In order, however, to make possible a comparison between the results of studies based on measurements of the transverse diameter and the data in our standard tables we have introduced a column showing the approximate transverse diameter corresponding to the silhouette areas given in tables A and B.

To determine the approximate transverse diameter corresponding to a heart silhouette area of a given size we have tabulated the various transverse diameters corresponding to a given area, reported by Dietlen in his study of the hearts of adult men and women, those reported by Veith in his study of the hearts of children, and the data obtained in our own x-ray studies of the heart in the living and those obtained from a study of cadavers. While the variations in the size of the transverse diameter corresponding to an area of a given size are considerable the formula $1.18\sqrt{area} = transverse\ diameter$ gives a fair general standard as the following examples may show (table 5).

In slender, youthful adults, especially during deep inspiration the long axis of the heart tends toward the vertical and hence the transverse diameter of the heart becomes relatively small. The large number of such individuals included in the series studied roentgenographically by me tends to make the transverse diameter of hearts with a silhouette area of from 90 to 125 square centimeters average below the figures called for by the formula given above and utilized in tables A and B. The average is also low in several of the older groups of children studied by Veith and in the cadavers of small slender individuals studied by me.

In order to test out the values of the transverse diameter given in tables A and B from the standpoint of body weight the following table has been prepared (table 6).

For the lighter weights, the number of observations are relatively few. Veith's supine individuals number 80. The male individuals sitting are of two groups; (1) is composed of

TABLE 5

Observed transverse diameter for a given area compared with he standard based on the formula transversed diameter = 1.18 area

AREA	CALCULATED TRANSVERSE DIAMETER	OBSERVED TRANSVERSE DIAMETER
sq. cm.	*cm.*	*cm.*
10	3.7	3.4 (foetus)
21	5.4	5.3 (infant cadaver)
30	6.5	6.4 (infant cadaver)
34	6.9	7.0 (infant cadaver)
49	8.3	7.7 (infant cadaver)
59	9.1	9.3 (child, C.R.B.)
		8.4, 9.3 (children, Veith)
70	9.9	8.8, 9.3, 9.8 (cadavers)
		9.0, 9.30, 9.45, 9.55, 9.65, 9.75, 10.2 (boys, Veith)
		9.5, (girl, Veith)
80	10.5	10.3 (cadaver)
		10.9 (male, Dietlen)
		9.35, 9.5, 10.15, 11.00 (boys, Veith)
85	10.8	10.7, 10.9 (cadavers)
		11.1 (woman, Dietlen)
90	11.2	11.1 (cadaver)
		11.2 (boy, Veith)
95	11.5	10.8, 11.4 (cadavers)
		10.6 (woman, C.R.B.)
		11.2, 11.9 (men, Dietlen)
		11.1, 11.3, 11.3, 11.7, 12.3 (women, Dietlen)
100	11.8	11.3 (man, C.R.B.)
		11.5 (woman, C.R.B.)
		11.5 (man, Dietlen)
		11.3, 11.7, 12.0, 12.2, 12.4, 12.8 (women, Dietlen)
105	12.1	12.4 (cadaver)
		12.3 (man, C.R.B.)
		10.9 (woman, C.R.B.)
		12.1, 12.6, 13.0 (women, Dietlen)
		11.6, 11.6, 12.3, 12.3, 12.4, 12.4, 12.8, 12.9 (men, Dietlen)
110	12.4	11.7, 11.9, 12.1 (men, C.R.B.)
		12.3, 12.4 (women, C.R.B.)
		11.3, 11.8, 12.6, 12.8, 13.1, 13.5, 13.8 (men, Dietlen)
115	12.6	11.3, 11.7, 12.0, 12.1, 12.1, 12.2, 12.3, 12.7, 12.8 (men, C.R.B.)
		12.0 (woman, C.R.B.)
		12.7, 12.8, 13.1, 14.0, 14.2, 14.3 (men Dietlen)
120	13.0	11.3, 11.5, 12.2, 12.3, 12.7 (men, C.R.B.)
		11 4, 12.4, 12.6, 12.8, 12.9, 13.0, 13.1, 13.2, 13.3, 13.6, 13.8, 13.8, 14.2 (men, Dietlen)
125	13.2	12.6, 12.6, 12.9, 13.2, 13.3 (men, C.R.B.)
		12.4, 13.7, 13.9 (men, Dietlen)
130	13.5	12.9, 13.2, 13.5, 14.0 (men, C.R.B.)
		13.8, 13.9, 13.9, 14.0 (men, Dietlen)
140	14.0	13.6, 14.8 (men, C.R.B.)
150	14.4	14.8, 15.1 (men, C.R.B.)
		14.7 (man, Dietlen)

25 individuals (orphans), 23 of whom are also in the supine list; (2) is composed of 25 healthy school boys. The female sitting group is composed of 25 individuals. The children in the groups studied by me number only 7 boys and 13 girls below 44 K. weight. The individuals studied by me above 44 K. weight number 188 men and 42 women with apparently normal hearts.

TABLE 6

Table showing the average transverse diameter corresponding to a given body weight as reported by various observers compared to the standard given in tables A and B

WEIGHT	AVERAGE TRANSVERSE DIAMETER										
	Standard	Bardeen, sitting		Veith				Dietlen, supine	Otten, supine	Claytor and Merrill, vertical	
				Supine	Sitting						
		Male	Female	Male	Male 1	Male 2	Female			Male	Female
kilos	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*	*cm.*
15–19	8.3*	8.9	8.9	8.75	8.1	8.7	8.1				
20–24	9.0	10.2	8.6	9.42	9.1	9.3	8.6				
25–29	9.6	9.4		10.0	9.2	9.4	9.4				
30–34	10.2	10.8		10.5	10.3	9.5	9.8				
35–39	10.7	10.2	10.4	10.57	9.8	10.6	9.8				
40–44	11.1		10.7					11.3	11.1		10.2
45–49	11.5	10.8	11.3					11.4	11.4		10.2
50–54	11.9	12.0	11.7					12.4	11.6		10.7
55–59	12.3	12.4	12.2					12.9	12.3	10.9	11.0
60–64	12.6	12.7	12.2					13.1	12.7	11.8	11.2
65–69	13.0	13.0	14.2					13.2	12.7	11.8	11.1
70–74	13.3	12.9	12.8					13.4	13.0	12.3	11.6
75–79	13.6	13.5						14.3	13.2	12.4	11.9
80–84	13.9	13.7						14.4		12.9	

* For 17 K.

The Dietlen list is based on a study of 156 adult men, that of Otten on a study of 100 adults, those of Claytor and Merrill on a study of 37 men and 54 women.

Veith's boys sitting on the whole correspond well with the standard adopted. Boys supine show in general a larger transverse diameter, the girls a smaller transverse diameter than the standard. Considering the relatively small number involved

and the great individual variations found in the transverse diameter the averages in my own cases correspond fairly well with the standard. Dietlen's cases studied in the supine position show a greater transverse diameter than the standard which is based on the sitting position. On the other hand, the individuals studied by Otten show an average difference of 0.43 cm. below the standard for each group. This is probably due in the main to the fact that the individuals were relatively slender, averaging 3.2 per cent above the normal height for the average weight at age thirty, as given in tables A and B. Yet Otten gives the position of the long axis of the heart in the individuals studied by him in the supine position as obliquely placed in 35 per cent of the cases, perpendicularly placed in 13 per cent and transversely placed in 52 per cent so that in over half the cases the transverse diameter should be relatively large. The individuals studied by Claytor and Merrill have unusually narrow hearts. The upright position chosen by these investigators may have tended to bring the heart into the vertical position but with due regard for this the hearts seem to average abnormally narrow.

In general it may be said that for the supine or prone position about 7 per cent should be added to the transverse diameter over the figures given in the standard table; for the standing position, about 4 per cent should be subtracted.

I have found in seven cases at the end of deep inspiration in the prone, sitting and standing positions that the transverse diameter averaged respectively 14.1, 13.1 and 12.6 cm. In these the average for the prone position was 7.6 per cent greater than for the sitting, 3.8 per cent less for the standing than the sitting position. In eight cases studied during normal respiration but at the height of inspiration in the sitting and prone positions, the average transverse diameter sitting was 13.3, prone, 14.3, or 7.5 per cent greater in the prone than in the sitting position. In deep inspiration the change in the transverse diameter corresponds with the change in the area of the heart silhouette but in quiet inspiration the heart is relatively broader in the prone than in the sitting position.

c. Heart weight and body weight

In dealing with the relation of heart silhouette area to body weight we are dealing with factors which can be objectively studied on a large number of individuals. The determination of the relation of the size of the heart silhouette to the volume and weight of the heart is not open to so direct a study in human beings. After death the heart can be weighed and the weight of the heart may be compared with the weight of the body but unless death has occurred from accident we are not likely to be dealing with normal conditions. Conclusions as to what obtains in the living must be cautiously applied from study of the dead.

The relations of the size of the heart to the size of the body in cadavers has been studied from various points of view. Among the chief contributions to the subject are those of Boyd ('61), who made an extensive study of the average weight of various organs, including the heart in relation to body weight and age; of Thoma ('82), who utilized mathematical theories of probability in a valuable analysis of his own data and that of other investigators in a study of the relation of the weight of the heart to body weight; of W. Müller ('83) who utilized extensive data in a study of the relation of heart muscle weight to the weight of the heart as a whole and of the relation of heart muscle weight to body weight, height and age; of Beneke ('78) who studied the volume of the heart substance from the standpoint of body length; of H. Vierordt ('90) who has summarized the work of previous investigators and added data of his own; E. Kress ('02) who studied the weight of organs in children; and of Greenwood and Brown ('13) who have applied modern mathematical methods to a study of a small but carefully selected material.

The studies of these and of numerous other investigators have shown that there is a close correlation between the size of the heart and the size of the body, due probably to the need of a given mass of heart muscle to pump the blood to a given mass of tissue. Greenwood and Brown conclude that the correlation between the weight of the heart and that of the whole body is not much less than 0.5 and that the weight of the heart can be

deducted from the weight of the body and kidneys by means of a linear equation with an average error of about 8 per cent. If the mean heart weight of the cases studied by these authors be divided by the mean body weight, the heart weight is found to be approximately 0.575 per cent of the body weight. Individuals show a variation from this mean per cent of body weight of about 25 per cent in either direction that is, from about 0.45 per cent of body weight to 0.70 per cent of body weight.

These figures probably very nearly express the average relative weight of the heart. Other investigators who have studied a greater number of individuals have furnished data which differ more or less widely according to the material studied and methods used. As a rule the data have been presented from the standpoint of average body weight and average heart weight for a given age. By dividing the one by the other one obtains a rough estimate of the proportion between heart weight and body weight for a given age. Data obtained in this way have led to somewhat divergent results as may be seen in table 7.

Boyd studied a large number of individuals at the Marylebone Infirmary and a smaller number at the Insane Asylum at Sommerset. The figures for the latter are placed immediately below those for the former for age groups above 30. It will be noted that in general the hearts studied by Boyd are heavy in relation to body weight, running from 60 to 80 per cent of the body weight instead of less than 60. There is no great difference between the relative size of male and female hearts but the hearts studied at Sommerset are notable relatively smaller in size than those studied at Marylebone. The figures from Thoma are based on a mathematical study of the average heart weight found by Caspar-Liman, Blosfeld, Reid, Peacock and Boyd for a given age combined with Thoma's study of the average body weight for corresponding ages. They show a low relative weight of the heart and indicate that the high relative weight shown in Boyd's figures is due largely to body emaciation. The hearts studied by W. Müller are from a more carefully selected material and average relatively smaller in size than those of Boyd, 0.604 per cent of the body weight in males; .594 per cent in females. The very

TABLE 7

Proportional heart weight at various ages as reported by several investigators

AGE	OBSERVER																			
	Boyd				Thoma	Müller				H. Vierordt				E. Kress						
	Male		Female			Male		Female		Male		Female		Male		Female				
	Number	Per cent body weight	Number	Per cent body weight	Estimate based on various data	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight			
Birth	44	.648	42	.572	0.625	23	0.62	14	0.63	62	0.76	59	0.80	3	0.66	6	0.89			
1 mo.	16	.594	21	.651		45	0.64	47	0.63	7	0.51	12	0.49	2	0.83					
2–3 mo.						50	0.58	52	0.61	30	0.48	33	0.38	6	0.68	7	0.65			
4–6 mo.	15	.605	24	.716	0.444					28	0.38	26	0.36	8	0.67	11	0.64			
7–9 mo.										29	0.40	18	0.29	7	0.64	9	0.65			
10–11 mo.	46	.516	40	.628		34	0.60	32	0.60	6	0.41	6	0.44	6	0.54	5	0.67			
1 yr.					0.412					15	0.46	18	0.42	4	0.59	7	0.58			
2 yrs.	34	.736	32	.711	0.422	34	0.62	42	0.62	45	0.47	52	0.53	12	0.60	4	0.60			
3 yrs.	27	.662	29	.716	0.417					31	0.52	37	0.50	10	0.58	7	0.61			
4 yrs.					0.40	16	0.58	19	0.59	32	0.53	18	0.55	10	0.64	8	0.61			
5 yrs.					0.382					24	0.51	30	0.55	8	0.54	7	0.60			
6 yrs.	27	.676	20	.592	0.383					7	0.48	17	0.56	7	0.65	7	0.64			
7 yrs.					0.392					18	0.47	6	0.48	7	0.61	5	0.64			
8 yrs.					0.402	15	0.62	18	0.56	3	0.44	11	0.58	5	0.80					
9 yrs.					0.417					6	0.46	4	0.62	5	0.65	2	0.66			
10 yrs.					0.433					10	0.51	3	0.54	5	0.60	2	0.58			
11 yrs.					0.452					12	0.52	5	0.47	2	0.49	2	0.60			
12 yrs.					0.474					1	0.34	1	0.40							
13 yrs.	21	.634	17	.706	0.481	9	0.60	10	0.55	8	0.50	2	0.46	2	0.63	3	0.54			
14 yrs.					0.490					8	0.58	8	0.50	4	0.61	1	0.60			
15 yrs.					0.500					9	0.48	11	0.65	5	0.62	35	0.52			
16 yrs.					0.481					11	0.51	10	0.63							
17 yrs.					0.471					17	0.51	16	0.52							
18 yrs.	18	.699	15	.762	0.474	23	0.55	13	0.50	20	0.46	25	0.49							
19 yrs.					0.474					23	0.51	15	0.53							
20 yrs.					0.476					15	0.51	30	0 48							

TABLE 7—Continued

AGE	OBSERVER																
	Boyd				Thoma	Müller				H. Vierordt				E. Kress			
	Male		Female		Estimate based on various data	Male		Female		Male		Female		Male		Female	
	Number	Per cent body weight	Number	Per cent body weight		Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight	Number	Per cent body weight
21 yrs.......					0.481					33	0.49	22	0.48				
22 yrs.......					0.483					27	0.50	21	0.48				
23 yrs.......					0.490					24	0.48	22	0.49				
24 yrs.......					0.495					30	0.46	22	0.49				
25–30 yrs....	58	.675	74	.654	0.502	73	0.58	45	0.50	30	0.46	27	0.49				
	46	.597	29	.608													
31–40 yrs....	118	.720	87	.682		70	0.56	59	0.52								
	59	.603	49	.470													
41–50 yrs....	137	.705	106	.706		84	0.59	69	0.56								
	76	.690	49	.676													
51–60 yrs....	119	.719	106	.764		87	0.62	61	0.59								
	42	.637	39	.710													
61–70 yrs....	126	.763	149	.758		88	0.64	83	0.64								
	39	.728	41	.716													
71–80 yrs....	100	.774	150	.786		64	0.64	61	0.67								
	21	.693	20	.612													
81–90 yrs....	24	.840	76	.806		11	0.75	12	0.69								
	7	.740	5	.655													

young and the older age groups show relatively larger hearts than the others. The Vierordt figures show relatively smaller hearts than the Müller groups but larger than the Thoma groups. In this connection it should be noted that like Thoma, Vierordt takes a theoretical weight for a given age and divides this by the average heart weight of hearts of individuals of that age. Vierordt bases his estimates of body weight on data

from Quetelet and Lorey. In Vierordt's tables of the weight of organs in the adults selected from various investigators to illustrate normal build ('06, p. 34, 35) the percentage of body weight given for the heart varies from .477 per cent to .633 per cent with an average (not weighted) of .558 per cent. The figures of Kress for children which are based upon the average body weight divided by the average heart weight for each age show a considerably higher relative heart weight than that given in Vierordt's tables. In part this is due to emaciation in the children studied by Kress but it is not due entirely to emaciation because in case of several of the Kress groups the average body weight is to be looked upon as normal.

If one bases his estimate of the relative size of the heart in the living upon the relative size of the heart in the dead, as done by Boyd, Müller and Kress one is apt to get too high a relative heart weight owing to the relatively large degree of emaciation in the dead. If one bases his estimate upon a division of the average heart weight of a group of individuals by a body weight assumed to be normal for such a group like Thoma and Vierordt he is apt to get too low a proportional heart weight because the heart weight compared with the 'normal' body weight is not the average of a normal group but of a more or less emaciated group. The mean between the two estimates will probably more nearly approach the normal relative heart weight than an estimate based on either method alone.

The following table (table 8) shows the relative weight of the heart for each age group as reported by W. Mueller, the relative weight of the heart based on normal body weight as given in Tables A and B and the mean between the two. Owing to the fact that the average height and average age for each group is not given in the Müller tables the estimates of normal weight for each group are necessarily somewhat rough.

This table shows that the heart is relatively much smaller for a given age group if the normal weight for the group be taken instead of the average weight of the group and that the difference in general is greater during childhood than in adult life. The mean between relative weight based on average weight

TABLE 8

Body weight, average weight of heart at various ages and relative heart weight after W. Müller, body weight normal for a given age, relative weight of hearts studied by Müller based on normal body weight and mean between relative body weight based on observed and that based on normal body weights

AGE	NUMBER OF INSTANCES	BODY WEIGHT	AVERAGE WEIGHT OF THE HEART	RELATIVE WEIGHT	ESTIMATED NORMAL WEIGHT	RELATIVE HEART WEIGHT BASED ON NORMAL WEIGHT	MEAN RELATIVE WEIGHT
1. Males							
			grams		*kilos*		
Premature births	42	1.15	7.06	0.00615			
Mature births	23	3.35	20.79	0.00620	3.2	0.0065	0.0064
1 month	45	2.52	16.19	0.00643	3.8	0.00426	0.0054
2– 6 months	50	3.49	20.13	0.00576	5.9	0.0034	0.0046
7–12 months	34	5.13	30.64	0.00597	8.2	0.0037	0.0047
2– 3 years	34	8.57	52.7	0.00615	12.2	0.0043	0.0052
4– 5 years	16	11.26	65.2	0.00580	15.5	0.0042	0.0050
6–10 years	15	16.63	103.6	0.00623	21.5	0.0048	0.00551
11–15 years	9	27.3	163.8	0.00600	37.0	0.0044	0.0052
16–20 years	23	43.2	236.9	0.00548	61.5	0.0038	0.0057
21–30 years	73	51.3	297.4	0.00580	62.5	0.0048	0.00525
31–40 years	70	51.6	289.6	0.00561	65.0	0.0046	0.0051
41–50 years	84	52.0	304.2	0.00585	70.0	0.0043	0.0051
51–60 years	87	55.3	340.8	0.00615	70.0	0.0049	0.0055
61–70 years	88	54.0	345.9	0.00640	70.0	0.0049	0.0057
71–80 years	64	52.7	335.5	0.00637	70.0	0.0048	0.0056
81–90 years	11	42.3	315.7	0.00746	70.0	0.0047	0.0061
2. Females							
Premature births	48	1.24	7.29	0.00587			
Mature births	14	3.06	19.24	0.00629	3.2	0.0060	0.0062
1 month	47	2.27	14.36	0.00632	3.8	0.0038	0.0051
2– 6 months	52	3.31	20.18	0.00610	5.9	0.0034	0.0048
7–12 months	32	5.34	32.14	0.00602	8.2	0.0039	0.0050
2– 3 years	42	7.34	45.2	0.00616	11.7	0.0039	0.0050
4– 5 years	19	11.67	69.0	0.00591	15.5	0.0044	0.0052
6–10 years	18	14.7	82.0	0.00561	21.5	0.0038	0.0047
11–15 years	10	32.2	177.4	0.00551	38.0	0.0047	0.0051
16–20 years	13	43.5	205.2	0.00495	54.5	0.0039	0.0044
21–30 years	45	46.2	220.6	0.00499	55.5	0.0040	0.0045
31–40 years	59	44.9	234.7	0.00523	58.5	0.0040	0.0046
41–50 years	69	47.1	264.1	0.00561	61.0	0.0043	0.0050
51–60 years	61	43.4	256.9	0.00592	61.0	0.0042	0.0051
71–80 years	61	44.1	294.3	0.00667	61.0	0.0048	0.0051
81–90 years	12	36.7	253.0	0.00689	61.0	0.0041	0.0055

and that based on normal weight runs between 0.5 and 0.55 per cent of the body weight for most groups in both the males and females but averages higher in the males.

At and immediately following birth most investigators have found that the heart is relatively large. If, however, the weight of the membranes at birth are included the proportional weight given above holds approximately true.

Thus Thoma estimates the normal average weight of the new born at 3.96 K. including membranes but not including the amniotic fluid and at 3.35 K. including the membranes. The heart he estimates at 0.532 per cent of the body weight if the membranes are included, at 0.625 per cent if they are not included.

The following table (table 9) from Müller indicates that similar relations prevail during the latter part of foetal life.

TABLE 9

Data from W. Müller on the relative weight of the heart in foetuses

(a) NUMBER	(b) LENGTH	(c) WEIGHT OF FOETUSES	(d) WEIGHT OF MEMBRANES	(e) WEIGHT OF HEART	$\frac{e}{c+d}$
	mm.	*grams*			
25	212	201	120	1.15	0.00354
16	330	783	232	4.44	0.00436
15	385	1296	315	8.08	0.00528
22	423	1727	394	10.74	0.00507
13	456	2252	487	13.81	0.00504
13	492	2756	501	18.68	0.00574
24	522	3448	572	21.36	0.00531

From the data given above we conclude that fifty-five hundredths per cent of the body weight approximates closely the normal relative proportions of the heart weight in males at all ages except at and immediately following birth and that in females the heart is slightly lighter, about fifty-three hundredths per cent of body weight. In our tables we have not, however, attempted to plot separate curves for males and females. The estimates of heart weight given in tables A and B are based upon the assumption that the weight of the heart is 0.55 per cent of the body weight. In estimating the relative weight at birth the weight of the foetal membranes is included in the body weight.

On testing this estimate on dissecting room material we have obtained the data shown at the right in table 4, p. 444. The bodies studied were all embalmed at the time of weighing but it is assumed that the ratio between heart weight and body weight was not thus markedly altered. The percentages of divergence given are the percentages above or below the standard adopted for the weight of a heart belonging to a body of a given weight. Thus for a body weighing 50 K. we should expect a heart weight of 275 gr. If the heart weighs 286 gr. we designate it + 4 per cent; if 264 gr. − 4 per cent. The average weight for twenty-five adult male hearts was 2.0 per cent below the standard, that of nine adult female hearts 1.1 per cent below the standard. A foetus and one new born child had hearts below the standard. One new born child had a heart considerably above the standard. Of four young children two had hearts below the standard; two had hearts heavier than the standard.

Into the weight of the heart there enter four main factors, (1) the heart muscle tissue, (2) the connective tissues of the valves and supporting structures, (3) the intrinsic blood vessels of the heart and the great vessels near their attachment to the heart, and (4) the fat deposited beneath the pericardium and elsewhere in the heart. Of these factors the heart muscle tissue is dynamically the most important and varies in amount with the dynamic demands on the heart. These demands to a large extent are determined by the weight of the body and hence the heat varies in size with body weight. The mass of the intrinsic blood vessels of the heart probably varies normally directly with the mass of the heart muscle. The great vessels near the heart are relatively heavier than one might estimate so that the relative weight of the heart found by different observers varies to some extent with the amount of great vessel tissue included with the heart. The fat likewise constitutes no inconsiderable part of the heart mass. To some extent it varies directly with the relative amount of fat in the body as a whole.

The two observers who have studied most carefully the relation of heart weight to body weight, Thoma and W. Müller, have excluded in some of their tables so far as possible the great

vessels and cardiac fat from the heart weight so as to get the proportion between the heart muscle tissue and the body weight. With the heart muscle tissue the connective tissue framework and the intrinsic blood vessels of the heart are, however, included. The following tables (tables 10 and 11) show the relation of the weight of the heart muscle tissue to body weight found by W. Müller:

From these tables it may be seen that the average percentage of body weight made by the heart muscle tissue in the males studied by W. Müller was 0.534 per cent; 52.8 per cent, in those above one year of age. In the females studied by W. Müller the average is slightly lower, 0.523 per cent; 0.50 per cent for those above one year of age. The range in the various weight groups is for males from 0.590 per cent for the 1–10 K. weight group to 0.391 per cent in the 100–110 K. group; for females from 0.584 per cent in the 1–10 K. group to 0.302 per cent in the 100–110 K. group. For each weight group Müller gives the average height and average age. In a subsequent section I give a brief description of statistical data relating to the normal weight for a given height and age. From the average height and average age for each weight group in the Müller table we may estimate a normal weight in contrast to the actual body weight. By dividing the average heart weight of each group by the estimated normal body weight we get the percentage of estimated normal body weight. This percentage indicates what the ratio of heart weight to body weight would be if the body weight were normal for height and age and the heart weight that actually found. This per cent of normal body weight is smaller than the per cent of actual body weight for the lighter groups, greater in the heavier groups, indicating that the majority of individuals in the former groups were underweight from the standpoint of height and age; those of the fatter groups overweight from the standpoint of height and age. The heart musculature is heavy compared with the body weight in thin individuals, light compared with the body weight in fat individuals.

By taking the mean between the heart weight-body weight ratio actually found and that which would have been found had

TABLE 10

Relative heart-muscle weight in males as reported by W. Müller compared with the relative weight when the body weight is normal for height and age

BODY WEIGHT	AVERAGE WEIGHT HEART	PER CENT BODY WEIGHT	NUMBER INDIVIDUALS	AVERAGE HEIGHT	AVERAGE AGE	NORMAL WEIGHT	PER CENT NORMAL WEIGHT	PER CENT OF MEAN WEIGHT
kilos					*years*	*kilos*		
1– 10	28.58	0.590	158	62.2	1	6.3	0.454	0.522
10.001– 20	81.2	0.535	40	104.4	7	17.1	0.473	0.504
20.001– 30	137.5	0.545	13	149.5	18	42.0	0.327	0.436
30.001– 40	199.6	0.562	98	160.6	48	62.5	0.319	0.441
40.001– 50	235.8	0.522	165	165.3	51	66.5	0.355	0.439
50.001– 60	276.0	0.505	127	167.2	50	67.5	0.409	0.457
60.001– 70	323.7	0.470	59	170.0	50	71.0	0.456	0.464
70.001– 80	360.8	0.488	23	169.8	53	71.0	0.508	0.488
80.001– 90	424.6	0.502	7	169.3	55	70.0	0.606	0.554
90.001–100	382.6	0.401	3	174.0	52	74.0	0.578	0.459
100.001–110	400.4	0.391	3	176.6	63	76.0	0.527	0.459
Weighted average.........		0.534					0.404	0.469
Weighted average above 1 year....................		0.528					0.390	0.459

TABLE 11

Relative heart muscle weight in females as reported by W. Müller compared with the relative weight when the body weight is normal for height and age

BODY WEIGHT	AVERAGE WEIGHT HEART	PER CENT BODY WEIGHT	NUMBER INDIVIDUALS	AVERAGE HEIGHT	AVERAGE AGE	NORMAL WEIGHT	PER CENT NORMAL WEIGHT	PER CENT OF MEAN WEIGHT
kilos					*years*	*kilos*		
1– 10	29.2	0.584	171	63.5	$1\frac{1}{3}$	6.6	0.443	0.514
10.001– 20	74.8	0.504	41	107.0	8	18.0	0.416	0.460
20.001– 30	139.6	0.554	20	144.3	18	44.0	0.317	0.436
30.001– 40	187.0	0.532	144	153.0	54	58.0	0.323	0.428
40.001– 50	224.5	0.499	137	156.3	51	60.0	0.374	0.437
50.001– 60	252.5	0.457	55	159.1	49	62.5	0.404	0.431
60.001– 70	270.3	0.420	28	161.1	51	64.7	0.418	0.419
70.001– 80	283.9	0.386	6	160.3	66	63.5	0.447	0.417
80.001– 90	227.8	0.280	1	162.0	23	56.6	0.402	0.341
90.001–100	363.6	0.400	1	166.0	64	66.5	0.547	0.473
100.001–110	316.6	0.302	1	163.7	46	65.5	0.483	0.393
Weighted average..........		0.523					0.388	0.455
Weighted average above 1 year....................		0.500					0.367	0.434

the body weight been normal for height and age we get the results shown in the column at the right in tables 10 and 11. The weighted average for males is 0.469 per cent, for males above one year of age 0.459 per cent. The weighted average for females is 0.455 per cent, for females above one year of age 0.434 per cent. In six of the weight groups of the males the per cent of mean body weight is less than 0.46 per cent, in five greater than 0.46 per cent. The highest percentage 0.554 per cent is found in the 80–90 K. group. The lowest 0.436 per cent in the 20–30 K. group. In eight of the weight groups of the females the percentage is below 0.46 per cent, in three 0.46 per cent or higher. The highest percentage, 0.514 per cent is in the 1–10 K. group, the lowest, 0.341 per cent, in the 80–90 K. group.

Thoma from a mathematical study of a less extensive material than that of Müller but carefully selected, found an average heart-muscle body-weight ratio of 0.463 per cent. This figure lies midway between the averages given above for the mean body weight in men and that in women. In round numbers we may take the heart muscle weight to be approximately 0.46 per cent of the body weight in individuals of normal build, slightly higher in men, slightly lower in women, higher in thin individuals, lower in fat individuals.

The Müller data arranged according to height (table 12) show the relative heart muscle weight ranging from 0.501 per cent to 0.532 per cent, in men, but this relatively high figure is due as pointed out above to the inclusion of a large proportion of relatively thin individuals.

According to Müller there is an increase in the relative weight of the heart with age irrespective of size of body as illustrated in table 13. However, it must be remembered that there is normally an increase in weight during adult life until old age comes on. The heart enlarges to meet this increase in weight. Sickness reduces the weight of the body usually more than the weight of the heart musculature so that after death the heart may appear relatively large in proportion to body weight.

The relative distribution of the musculature within the heart has been studied by several investigators among whom the work

TABLE 12

Relative heart muscle weight in individuals grouped according to height after W. Müller

BODY LENGTH	NUMBER OF INDIVIDUALS	ABSOLUTE HEART WEIGHT	RELATIVE HEART WEIGHT
	Men		
1501–1550	27	228.5	0.00532
1551–1600	62	219.1	0.00502
1601–1650	115	227.6	0.00528
1651–1700	89	216.9	0.00501
1701–1750	62	222.0	0.00519
1751–1800	29	224.9	0.00522
	384		
	Women		
1401–1450	21	213.3	0.00495
1451–1500	55	213.4	0.00488
1501–1550	98	210.1	0.00487
1551–1600	93	200.1	0.00455
1601–1650	51	204.5	0.00498
1651–1700	25	204.5	0.00465
1701–1750	9	208.9	0.00497
	352		

TABLE 13

Relative heart muscle weight in adults grouped according to age after W. Müller

AGE	MALES		FEMALES	
	Absolute heart weight	Relative heart weight	Absolute heart weight	Relative heart weight
20–40	243.3	0.00493	190.6	0.00432
40–60	265.0	0.00499	216.6	0.00473
60–80	274.9	0.00513	240.9	0.00523
Over 80	258.9	0.00606	203.9	0.00539

of W. Müller seems to have been the most extensive. He found the musculature of the left ventricle to weigh approximately twice that of the right ventricle and the musculature of auricles to weigh approximately one-fourth that of the ventricles. The weight of the values of the heart is about two per cent of the weight of the heart as a whole.

Taking the relative weight of the heart musculature to be 0.46 per cent of the body weight we may next consider the weight of the non-muscular structures. Of the non-muscular structures fat constitutes the chief mass after early infancy. The most careful study of the amount of fat in the heart is that of W. Müller. He has shown that amount of fat in the heart increases with age. In the new born there is relatively little but later in life it constitutes no inconsiderable part of the weight of the heart. Part of the fat is as a rule easily removed with the pericardium but about 8 per cent can be removed only when special methods are used. The average amount of fat may be illustrated by the following table based on data from Müller (table 14).

The body weight is estimated from data given in table 8.

The relative amount of fat may be judged by comparing the weight of the heart as a whole at various ages with the weight of the heart muscle as shown in the following table (table 15).

The data concerning the weight of cardiac fat relative to body weight given in table 14 do not quite correspond with the data of table 15, owing probably to some variation in the individuals composing the various age groups but the differences in the two tables are not serious. To some extent, at least, the greater amount of fat found by Müeller in the older age groups is due to the greater emaciation of the younger as compared with the older individuals studied by him. A rough estimate of the degree of emaciation may be made by comparing the average body weight for each of the Müller age groups with the body-weight estimated as normal for a given age and height in tables A and B. This comparison shows that while the new born in Müller table are of about normal weight male infants during the first year of life are nearly 50 per cent underweight and during the second and third years about 33 per cent underweight. After this period the average body weight for each age group appears to be only from 25 to 30 per cent under the weight of healthy living individuals during youth, 20 to 25 per cent during adult life until the oldest age period when the average body weight seems to be 40 per cent underweight. The female infants appear to be 4 to 50 per cent underweight, girls up to ten

TABLE 14

Amount of fat in the heart at various ages after W. Müller

AGE	NUMBER INDIVIDUAL	AVERAGE WEIGHT OF HEART	PERICARDIAL FAT IN GRAMS			MAXIMUM	MINIMUM	PER CENT OF HEART WEIGHT	PER CENT OF BODY WEIGHT
			Removable	Resistant	Total				
1. Males									
		grams							
Premature births	42	7.06	0	0	0	0	0	0	0
Mature births	23	20.79	0	0	0	0.1	0	0	0
1 month	45	16.19	0		0	0	0	0	0
2 months	14		0.036	0.039		0.510	0.000		
3– 4 months	22	20.13	0.185	0.015	0.200	0.520	0.000	1.6	0.009
5– 6 months	14		0.449	0.036	0.485	2.10	0.00		
7–12 months	34	30.64	0.998	0.079	1.077	3.10	0.00	3.5	0.021
2– 3 years	34	52.7	2.89	0.23	3.12	8.10	.00	5.9	0.036
4– 5 years	16	62.5	5.81	0.42	6.23	9.46	2.57	10.0	0.054
6–10 years	15	103.6	9.2	0.7	9.9	13.7	1.8	8.6	0.060
11–15 years	9	164.8	14.7	1.2	15.9	16.8	8.0	9.7	0.058
16–20 years	23	236.9	27.8	2.2	30.0	72.4	15.5	12.7	0.070
21–30 years	73	297.4	31.4	2.5	33.9	77.8	0.0	11.4	0.066
31–40 years	70	289.6	36.1	2.9	39.0	90.7	9.3	13.8	0.076
41–50 years	84	304.2	46.2	3.7	49.9	240.4	9.7	16.4	0.096
51–60 years	87	340.8	55.2	4.4	59.6	266.2	13.0	17.5	0.108
61–70 years	88	345.9	59.2	4.7	64.1	159.2	5.2	18.5	0.119
71–80 years	64	335.5	64.6	5.2	69.8	169.4	9.4	20.8	0.132
81–90 years	11	315.7	58.0	4.6	62.6	146.7	47.9	19.8	0.148
2. Females									
Premature births	48	7.29	0	0	0	0	0	0	0
Mature births	14	19.24	0	0	0	0.3	0	0	0
1 month	47	14.36	0	0	0	0	0	0	0
2 months	14		0.105	0.008	0.113	1.10	0	0.5	
3– 4 months	28	20.18	0.138	0.011	0.149	1.60	0	0.74 1.2	0.007
5– 6 months	10		0.634	0.051	0.685	1.63	0	3.4	
7–12 months	32	32.14	1.37	0.109	1.479	3.70	0	4.1	0.028
2– 3 years	42	45.2	3.09	0.25	3.34	7.03	0	7.3	0.046
4– 5 years	19	69.0	5.86	0.47	6.33	17.1	0	9.4	0.054
6–10 years	17	82.5	9.18	0.73	9.91	17.3	2.2	12.0	0.067
11–15 years	10	177.4	16.3	1.3	17.6	15.3	5.8	9.9	0.055
16–20 years	13	215.2	23.2	1.8	25.2	42.2	4.0	11.6	0.060
21–30 years	45	220.6	30.2	2.4	32.6	74.2	3.9	15.2	0.070
31–40 years	59	234.7	38.2	3.0	41.2	85.0	10.9	17.6	0.092
41–50 years	69	264.1	45.9	3.7	49.6	104.3	11.0	18.5	0.105
51–60 years	61	256.9	44.2	3.5	47.7	115.5	9.1	19.3	0.110
61–70 years	83	285.1	52.2	4.2	56.4	192.0	19.4	19.8	0.127
71–80 years	61	294.3	56.4	4.5	60.9	179.1	14.4	20.7	0.138
81–90 years	12	253.0	49.1	3.9	53.0	79.7	25.8	20.9	0.145

TABLE 15

Relative weight of the heart, of the heart musculature, and of cardiac fat after data from W. Müller

AGE	NUMBER INDIVIDUALS	RELATIVE WEIGHT OF HEART	RELATIVE WEIGHT OF MUSCULATURE	RELATIVE WEIGHT OF CARDIAC FAT
Males				
		per cent	*per cent*	*per cent*
Birth	23	0.620	0.620	0.000
1 week	18		0.645	
2 weeks	13	0.643	0.627	0.016
3 weeks	10		0.655	
4 weeks	5		0.645	
2 months	15		0.590	
3 months	14	0.576	0.563	0.013
4–6 months	24		0.557	
7–12 months	34	0.597	0.580	0.017
2 years	17	0.615	0.557	0.058
3 years	13		0.522	
4–5 years	16	0.580	0.493	0.087
6–10 years	16	0.623	0.542	0.081
11–15 years	8	0.600	0.514	0.086
16–20 years	23	0.548	0.491	0.057
21–30 years	69	0.580	0.500	0.080
31–40 years	69	0.561	0.486	0.075
41–50 years	84	0.585	0.494	0.091
51–60 years	87	0.615	0.504	0.111
61–70 years	87	0.640	0.522	0.118
71–80 years	63	0.637	0.504	0.133
81–90 years	11	0.746	0.606	0.140
Females				
Birth	14	0.629		
1 week	18		0.624	
2 weeks	13	0.632	0.652	
3 weeks	10		0.578	
4 weeks	5		0.649	
2 months	15		0.613	
3 months	14	0.610	0.583	0.027
4–6 months	24		0.582	
7–12 months	34	0.602	0.570	0.032
2 years	17	0.616	0.572	0.046
3 years	13		0.510	
4–5 years	16	0.591	0.522	0.069
6–10 years	16	0.561	0.497	0.064
11–15 years	8	0.551	0.461	0.090
16–20 years	23	0.495	0.441	0.054
21–30 years	69	0.499	0.432	0.067
31–40 years	69	0.523	0.431	0.092
41–50 years	84	0.561	0.460	0.101
51–60 years	87	0.592	0.486	0.106
61–70 years	87	0.641	0.489	0.152
71–80 years	63	0.667	0.558	0.109
81–90 years	11	0.689	0.539	0.150

years about 33 per cent underweight, the older children and women 2 to 25 per cent underweight, the oldest age group 40 per cent underweight.

In the males the relative weight of the cardiac fat compared with the body weight averages about 0.077 per cent from the second to the fiftieth year with 0.057 per cent as the minimum for an age group, 0.091 per cent as the maximum. Below the second year, due in part at least to emaciation, the percentage is markedly less, after the fiftieth year, due in part to a relatively large amount of general body fat, it is greater. In the females the percentage of cardiac fat averages 0.073 per cent from the second to the fortieth year. It is only 0.046 per cent in the second year and is markedly less in the younger infants. It is 0.092 per cent in the 31 to 40 year age group and from 0.101 per cent to 0.152 per cent in the older age groups.

We may therefore assume that 0.08 per cent of the body weight represents approximately the proportional amount of cardiac fat except in early infancy, when it is less and after fifty when it becomes greater.

The intrapericardial part of the great vessels of the heart makes up a much less important part of the weight of the whole heart. The following table (table 16) based on data from W. Müller shows that the intrapericardial part of the chief of these vessels, the aorta and pulmonary artery weigh about 0.005 per cent of the body weight and that they weigh relatively somewhat more in old age than in youth. The percentage of body weight is based upon the average body weight given by Müller for the age groups in which he has studied the heart weight (table 8).

d. Heart volume

Estimation of the normal ratio between heart weight and body weight while unsatisfactory because based in the main on bodies made abnormal by disease has the advantage of a basis of direct observation. Estimation of the normal volume of the human heart filled with blood in the living can be arrived at only indirectly. We may estimate it either from the size of the

TABLE 16

Weight of the intrapericardial part of the great arteries after W. Müller

AGE	SEX	NUMBER OF OBSERVATIONS	WEIGHT OF INTRAPERICARDIAL PART OF GREAT ARTERIES IN GRAMS			PROPORTION OF BODY WEIGHT
			Medium	Maximum	Minimum	
Embryos	M	19	0.55	1.34	0.09	
	F	19	0.59	1.55	0.09	
1 year	M	41	1.80	3.30	0.77	0.004
	F	31	1.54	3.50	0.62	0.003
2 years	M	6	2.72	4.50	1.75	0.004
	F	13	2.87	4.40	2.11	
3 years	M	4	4.5	5.5	4.0	0.005
	F	4	4.2	5.0	3.6	
4 years	M	3	4.6	5.5	4.0	
	F	3	4.1	4.8	3.5	0.004
5 years	M	1	4.3			0.005
	F	2	5.5	5.7	5.2	
6–10 years	M	3	7.0	7.2	6.8	0.004
	F	6	5.9	8.7	3.8	0.004
11–15 years	M	2	8.5	22.0	8.5	0.003
	F	1	6.2			
16–20 years	M	7	14.7	22.0	11.5	0.003
	F	6	11.8	14.2	10.0	0.003
21–30 years	M	18	20.5	43.0	13.5	0.004
	F	16	14.9	25.2	11.0	0.003
31–40 years	M	14	20.3	25.0	15.0	0.004
	F	26	17.9	30.3	11.0	0.004
41–50 years	M	26	25.9	44.0	15.0	0.005
	F	24	23.9	47.0	15.2	0.005
51–60 years	M	23	27.8	41.0	17.5	0.005
	F	13	22.4	29.8	15.0	0.005
61–70 years	M	30	31.4	56.0	23.0	0.006
	F	24	26.6	41.5	18.0	0.006
71–80 years	M	19	32.5	49.0	22.5	0.006
	F	21	27.7	40.0	17.0	0.006
81–90 years	M	4	28.9	31.0	27.0	0.007
	F	5	30.2	47.2	21.0	0.0082

area of the heart silhouette or from the ratio between heart weight and body weight and that between heart volume and content volume.

If the heart in the living always had exactly the same shape and if it were so placed so as to obstruct the x-rays in a uniform

manner all that would be necessary to determine the heart volume from a given silhouette would be the knowledge of a constant to multiply into cube of a given diameter, just as we may quickly estimate the volume of a sphere from a knowledge of π and the radius of the sphere. The hearts of different individuals differ somewhat in shape and vary also under different conditions in the same individual. Furthermore it is difficult to place two different individuals so that the heart will obstruct the x-rays in an exactly equivalent manner. The best we can do is to use methods which will give approximately equivalent heart silhouettes and make use of a formula for volume which will give approximate volume.

After considerable experimenting not only on the living but on cadavers the position described above, sitting down, leaning forward, and with the breath held at the end of a moderately deep inspiration, was selected as that giving the most uniform heart-silhouette with relation to heart volume. Study of the volume of moderately distended hearts in cadavers in relation to the areas of heart outlines drawn as described above so as to correspond with the silhouette areas of x-ray plates led to the establishment of the following formula for heart volume based on silhouette area:

$$0.53\ A^{3/2} \qquad A = \text{silhouette area}$$

The estimates of heart volume given in tables A and B are based upon this formula. For the sake of convenience the volume is given in round numbers in cubic centimeters. While there is no direct method of testing the accuracy of this formula in the living, dissecting room data may prove of interest in showing how closely area and volume correspond. The method of estimating the area in the dissecting room has already been described, p. 438. The volume is determined by plugging the orifices of the heart and then measuring the amount of water or oil displaced by the heart. If the cavities of the heart are empty they are filled with the fluid used for measuring the displacement before the orifices are plugged. The aorta and pulmonary artery are included in the volume to a level which

corresponds with the arbitrary line selected for demarcating the base of the heart silhouette area.

The following table, table 17, shows the ratio of the observed to the volume estimated from silhouette area in sixty-two bodies. The difference between the observed and calculated volume and the percentage of divergence from the calculated volume were

TABLE 17

Relation of observed volume to volume estimated from silhouette area and of observed heart weight to weight estimated from silhouette area

RELATION VOLUME TO ESTIMATED VOLUME			PER CENT DIVERGENCE WEIGHT OF HEART FROM ESTIMATED WEIGHT										EXTREMES OF VOLUME
			Weight underestimated						Weight overestimated				
Number cases	Sex	Per cent Divergence	Number cases	+ 20	+ 15	+ 10	+ 5	+ 2½ to − 2	− 5	− 10	− 15	− 20	
Volume underestimated													
2	M		2	1								1	490–990
1	F	+20	1	1(31.2)									510
5	M		4	1(48.6)		1	1	1					210–525
1	F	+15	1					1					410
2	M	+10	2						1	1			600–685
9	M		6		2	1	1	1	1				287–1000
1	F												
1	G	+ 5	1			1							55
22	18M 3F 1G		17	3	2	3	2	3	2	1		1	
Volume as estimated													
8	M		6	1(37.8)	1			2		2			350–790
5	F	+ 2	5						2	2		1	290–400
3	B	to − 2½	3							2		1(41.5)	15–105
16	8M 5F 3B		14	1	1			2	2	6		2	

TABLE 17—Continued

Volume overestimated													
7	M		6			1		1	1		1	2(1–30)	300–810
1	F		1									1	510
1	B	− 5	1					1					16
6	M		6	1(63.0)			1	1	1		2		320–700
1	F	−10											475
4	M		4								1	3(25, 31, 33.5)	390–1155
1	B	−15	1									1(36.7)	75
2	M		2									2(21.8, 34.2)	560–600
1	F	−20	1								1		290
24	19M 3F 2B		22	1		1	1	3	2		5	9	
			Weight underestimated						Weight overestimated				
			53	5	3	4	3		6	7	5	12	
				15					30				
			Weight correctly estimated										
			8										

determined for each heart. The hearts were then grouped according to the extent of divergence of observed from calculated volume. Hearts showing a divergence of $2\frac{1}{2}$ per cent or less are classed together. The others are classed to the nearest 5, 10, 15 or 20 per cent + or −. Markedly distorted hearts have been excluded but no attempt has been made to select hearts that conform to theory. For the sake of comparison the divergence of observed heart weight from the heart weight calculated from silhouette area is likewise given.

This table shows that of the 62 hearts included in the study the volume was correctly calculated from the area to within $2\frac{1}{2}$ per cent in 16 cases, to within the nearest 5 per cent + or − in 36 cases and to within 10 per cent (the nearest 10 per cent + or −) in 45 cases. It is probable that greater accuracy can be obtained in estimating heart volume from silhouette area in

the living than in the dead since the condition of the heart with relation to the distention of its chambers is more uniform in the living. We believe that the formula given above enables one to calculate diastolic volume from silhouette area to within 5 per cent of the volume in diastole in the majority of instances in the living.

In cadavers there is a tendency to underestimate volume from silhouette area when the heart is contracted; to overestimate volume when the heart is more distended than is normal in diastole. Whether or not this is true in the living we have no means of ascertaining at present. If we take the heart weight considered standard for a person of a given body weight as described in Section C, p. 449—and given in tables A and B, and the silhouette area considered standard for a given body weight as described in Section A, p. 431, and given in tables A and B we see that there is a constant relation between silhouette area and heart weight if each is assumed to bear a constant relation to body weight. We may express this relation by the formula:

$$\tfrac{1}{20}\ \text{area}^{3/2} \times 0.0055 = \text{heart weight}.$$

The area is here assumed to be the area in square centimeters of the heart silhouette in diastole while sitting at rest and the heart weight that of the whole heart in grams. If the heart is more contracted than is normal in diastole when the body is sitting at rest the weight of the heart in relation to the silhouette area is increased. If the heart is more dilated than is normal for this position the weight of the heart in relation to the silhouette area is decreased. We thus have a method of determining in a more or less rough way whether or not a heart in the cadaver is more or is less dilated than is normal in diastole when the body is at rest in the living. In table 17 the percentage of divergence of the observed from the heart weight estimated from silhouette area is given for 53 of the 62 bodies in which the relation of silhouette area to volume was studied. From this table it may be seen that of the 17 hearts whose volume was underestimated from the shadow area, ten were underestimated from the standpoint of weight and four overestimated. This

would indicate that there is a tendency to underestimate volume from silhouette area when the heart is contracted. On the other hand of the 22 bodies in which the volume was overestimated from the shadow area, the weight was overestimated in 16 and underestimated in 3 indicating that these hearts were more dilated than is normal for diastole at rest. This same condition is, however, also true of the hearts in which the observed volume fairly closely corresponded with the estimated volume. Of the fourteen hearts in this group in 10 the weight was overestimated, in 2 underestimated.

Of the total of 53 hearts studied in 15 the weight was underestimated from the silhouette area, in 30 overestimated. We may therefore assume that the method used in preparing the bodies tended in the main to cause a somewhat greater distention than is normal in diastole in the living under the conditions described above.

We may likewise estimate heart volume from heart-weight, which we have assumed to be 0.55 per cent of the body weight. To determine a formula to express the relation of heart weight to diastolic heart volume we need to know the relation of heart weight to heart tissue volume and the relation of the volume of heart tissue to the volume of the heart and its contents in diastole.

In order to estimate tissue volume from heart weight we have to determine the specific gravity of the heart. Vierordt, quoting Davy, gives 1049 as the specific gravity of the left ventricle. I have estimated the specific gravity of a considerable number of fresh dog hearts, of one unembalmed human heart and of numerous embalmed human hearts. The method used was to measure the displacement of the heart in oil and to estimate the specific gravity from this. The heart was in each case freed from extraneous substances but the subepicardial fat was left in place. The chief difficulty met with was to get rid of air bubbles. To aid in this the heart was cut into sections. For exact work the displacement should be measured in a vacuum but this was deemed unnecessary for the purpose in view. While there were individual variations, due chiefly to differences in the amount of subepicardial fact, the figure 1050 was selected as a

round number which expressed with fair accuracy the specific gravity of the heart as a whole.

The volume of the empty heart in centimeters may therefore be taken as equal to the weight of the heart in grams divided by 1050. The ratio between the volume of the empty heart and that of the heart in diastole can be estimated from cadavers and from experimental work on animals. The chief difficulty lies in the determination of the volume of the heart in diastole.

In order to determine the ratio between the volume of the empty heart in dogs and the volume of the heart in diastole, I have made a number of experiments in coöperation with Dr. J. A. E. Eyster and other members of the department of physiology at the University of Wisconsin. The dog was weighed and its pulse at rest under morphine was counted before beginning the experiment. The animal was then anaesthetized, the thorax opened and ligatures were placed about each of the vessels entering the heart. With the help of several assistants these ligatures were tightened simultaneously at a given signal so as to close off the vessels during diastole. The heart was now removed from the body and its volume estimated. It was emptied and the volume of the cardiac tissue was measured and its weight determined. The ratio of the volume of the empty heart to that of the heart in diastole could then be ascertained. In order to make the pulse correspond approximately with the normal pulse as determined before the experiment, or somewhat slower, the vagus nerve was stimulated during the experiment to the requisite amount. The chief difficulty in the experiment is that of tying off all the vessels simultaneously at the height of diastole.

Table 18 shows the result of six experiments. The percentage of the diastolic heart volume occupied by the blood in the heart chambers varied from 26 to 46 with an average of 40.6. It is probable that the smaller percentage represents a heart in which we did not succeed in tying off all the vessels in diastole. If we omit this heart the average becomes 43.5. The average empty heart volume in these dogs was therefore 59.4 per cent if experiment 5 is included, 56.5 per cent if this experiment is not

included. It is of interest to note that the heart of the dog weighs more in relation to body weight than the human heart does and is subject to wider variations. This is in accord with the observations of Joseph ('08).

In the human heart the percentage of the diastolic heart volume occupied by the blood in the cavities appears to be greater than in the dog, the percentage occupied by the heart muscle less. In the study of embalmed cadavers the empty heart volume was found to vary from 33.8 per cent to 80 per cent of the volume of the heart as a whole. The hearts, the outline of which seemed most closely to correspond with radiographic outlines of

TABLE 18

Relation of diastolic volume to the volume of the empty heart in the dog

	WEIGHT OF DOG	PULSE RATE	WEIGHT OF HEART	PER CENT OF BODY WEIGHT	DIASTOLIC VOLUME	VOLUME EMPTY	PER CENT OF DIASTOLIC VOLUME	VOLUME OF BLOOD	PER CENT OF DIASTOLIC VOLUME
	kilos		*grams*						
1	10.4	80	107.7	1.0	176.5	102.6	58.1	73.9	41.9
2	12.0	96	84.0	0.7	135.0	80.0	59.2	55.0	40.8
3	10.2	110	57.1	0.56	106.0	60.0	56.6	46.0	43.4
4	13.0	75	112.0	0.86	195.0	106.7	54.7	88.3	45.3
5	8.36	120	70.0	0.84	90.0	66.7	74.0	26.0	26.0
6	16.0	120	135.5	0.847	238.9	129.0	54.0	109.9	46.0
Average				0.801			59.4		40.6

the living diastolic heart, showed an average percentage of about 49.4 per cent heart tissue, 50.6 per cent heart chamber space. If the specific gravity of the heart be taken as 1050 and the percentage of diastolic heart volume occupied by heart tissue be taken as 49.4 per cent we may estimate diastolic heart volume from heart weight by dividing the latter by 1.050×49.4 or 0.5187. The results thus obtained may be compared with the estimates given in tables A and B, in which the heart weight is estimated from the body weight and this in turn from the silhouette area while the volume is estimated directly from the silhouette area. The use of round numbers in the tables gives rise to slight divergencies but otherwise the estimates of heart volume based on silhouette area and those based on heart weight correspond.

The ratio between heart volume calculated from heart weight in bodies studied in the anatomical laboratory and the measured heart volume is shown in table 19. The hearts are grouped according to the extent of divergence of the observed from the calculated volume. Those showing less than $2\frac{1}{2}$ per cent of divergence are grouped together. The rest are grouped according to the nearest 5, 10, 15, 20, 25, 30, 35 and 45 per cent of

TABLE 19

Hearts from cadavers grouped according to percentage of divergence from the assumed ratio between empty heart volume and diastolic volume

PERCENTAGE OF DIVERGENCE	NUMBER OF CASES	SEX		EXTREMES OF HEART VOLUME		AVERAGE PER CENT OF DIVERGENCE FROM "NORMAL" HEART WEIGHT BODY WEIGHT RATIO
		Male	Female		Number cases	
				cc.		
+45	1	1		562	1	− 5.2
+35	1	1		470	1	−13.2
+30	2	2		950–990	1	−11.6
+25	1		1	365	1	−15.0
+20	7 (1 child)	6	1	75–687	6	+ 0.9
+15	4 (1 child)	3	1	105–1000	4	+ 3.2
+10	6 (1 child)	4	2	70–790	6	−12.2
+ 5	5 (1 foetus)	2	3	290–453	4	− 0.5
+ $2\frac{1}{2}$ to −$2\frac{1}{2}$	10 (1 child)	8	2	167–810	8	− 3.2
− 5	5	5	0	210–700	7	− 0.6
−10	3	3	0	360–1155	2	+ 4.5
−15	2	2	0	350–420	2	− 3.9
−20	4 (1 infant)	3	1	15–520	3	− 9.4
−25	1	1	0	320		
Total........	52	41	11		46	

positive or negative divergence. Separate columns show the number of males and females in each group, the extremes of heart volume (including contents of chambers) and the average per cent of divergence from the 'normal' heart weight-body weight ratio of those of each group for which records were preserved.

From this table it may be seen that while the greatest number (10) of hearts fall within the group assumed to show normal

diastolic volumes ($+2\frac{1}{2}$ to $-2\frac{1}{2}$ per cent divergence), the number (27) of those which show a volume above what is assumed to be the normal diastolic volume is greater than the number (15) which show a volume smaller than the normal diastolic volume. This is what we should expect from the conditions of the hearts studied.

The post-mortem condition of the heart has been studied by several investigators including MacWilliam ('01) and Rothberger ('04). At the time of death the heart is in diastole. The amount of blood in the heart depends on the general circulatory conditions at this time. After death there is a tonic contraction of the heart followed by a rigor mortis contraction. The post-mortem contraction of the heart is usually much greater in individuals in whom the respiration stops before the circulation than in those in whom heart failure is a primary cause of death. The postmortem contraction is followed by a subsequent dilatation but the extent of this depends to a large extent on the amount of fluid blood under pressure when the dilatation occurs.

The bodies received at the Anatomical Laboratory at the University of Wisconsin have usually been dead at least a week. As a rule they are embalmed by injecting equal parts of alcohol, glycerine and carbolic acid into the femoral arteries and the thorax is not opened until the body is dissected. In some instances we have opened the thorax in order to study the condition of the heart before embalming. As a rule the right atrium is fairly well distended with blood and frequently there is considerable blood in the right ventricle. While there is usually some blood in the left atrium this is less apt to be distended than the right atrium. The left ventricle is usually practically empty. When the embalming fluid is injected under a pressure of five or six pounds into the femoral arteries it usually enters the chambers on the left side of the heart and distends them to a moderate degree. The right side of the heart is less affected by the injection than the left side. The embalming fluid is usually followed by a shellac and Prussian blue arterial injection mass which also usually partially fills the chambers in the left side of the heart but not those on the right side. We have not meas-

ured the pressure of the fluid in the heart at the time of embalming but it is probably considerably higher than the pressure in the heart during life at the beginning of systole. When the injection is completed both the right and left sides of the heart are probably as a rule more distended than is normal during life; the right as a result of natural factors active just before and following death, the left as a result of the pressure of the embalming fluid. The embalming fluid causes some shrinkage. The end result appears to be in many cases a heart having approximately the size of the living heart in diastole during bodily rest. The dilatation of the various chambers is probably seldom quite the same in the cadaver heart as in the living but the heart as a whole frequently appears not dissimilar in outline. If there has been an antemortem acute dilatation of the heart or if the embalming fluid causes unusual distention we may have a heart large in proportion to the weight of its component tissue. If less blood than usual is sent into the right side of the heart before death or if the distention of the heart by the embalming fluid is less than usual or the shrinkage greater the size of the heart in relation to the weight of its component tissue is relatively small.

The table shows that no clear relation exists between the weight of the heart compared to the weight of the body and the cadaver size of the heart compared with the weight of the empty heart.

The best estimate which we can make of the ratio of heart substance to heart content is on the one hand from the heart-weight-body weight ratio based on post mortem studies, on the other hand from the heart-silhouette area-body-weight ratio based on x-ray studies of the living. But it is of interest to see how closely the estimates thus made are approached by direct studies on the hearts of embalmed cadavers as shown in table 19.

e. Ventricular output

The chief interest in arriving at an approximate knowledge of heart content in diastole is in relation to the systolic output of the heart. Various methods have been used to determine the

amount of blood discharged from the heart at each systole. While the results have been far from uniform the results of the more recent work including that of Krogh and Lindhard ('12) and Lindhard ('15) appear to indicate that the output of the human adult heart at rest is not far from 1 cc. per kilo of body weight per beat. Since the weight of the heart substance may be estimated at 5.5 gr. per kilo, its specific gravity as 1050 and its volume at about 49.4 per cent of the volume of the heart in diastole the volume of heart content in diastole may be estimated as 5.365 cc. per kilo. About 20 per cent of the blood in the heart in diastole is thus sent into the aorta at each systole during rest. If we estimate one-third of the blood in the heart during diastole to be contained in each ventricle and one-third in the two atria we have 60 per cent of the contents of the left ventricle sent into the aorta at each systole during bodily rest.

In the upright position the diastolic heart is smaller than in the sitting position and in the sitting position than in the prone position. It appears that to the lessened hydrostatic pressure in the inferior vena cava and to the moderate exertion accompanying sitting and standing the heart accommodates itself by beating faster, contracting more completely during systole, and expanding less during diastole. Nicolai and Zuntz have shown, however, ('14) that during severe exercise the heart expands more during diastole than when at rest. Muscular action acts as a pump to force blood toward the heart. In all probability the heart also contracts more completely so that the output of the heart is increased by pulse volume as well as by pulse rate. The experimental work of Henderson and Barringer on the dog which has led these investigators to opposite deductions does not seem to me at all conclusive.

In order to test the estimate of heart content in diastole given above and to estimate the reduction in size of the heart during systole we have devised with the collaboration of Dr. J. A. E. Eyster, an apparatus for taking 'instantaneous' radiographs of the heart at any desired period of the cardiac cycle. The mechanism is adjusted to the carotid pulse. As a rule two successive radiographs are taken on the same plate, one at the

height of systole, one in diastole and the outlines of the two superimposed shadows are compared. The pictures are taken at the usual distance of two meters. Two intensifying screens are used, one on each side of a photographic film. Drum tracings of the respiration, carotid pulse and of the period of exposure are made while the pictures are taken. The estimates of change of heart volume from diastole to systole based on these plates correspond well with the data given above as the following table will show (table 20).

In the sitting position observations were made on sixteen individuals. For one individual two sets of observations are recorded in the table. During the change in heart volume from diastole to systole blood from the ventricles is forced into the pulmonary artery and aorta. Since the systolic picture was taken as nearly as possible at the height of ventricular systole it is possible that in most cases diastole had already begun in the atria and some new blood had entered these chambers. The actual output of the heart may therefore have been somewhat greater than that estimated from the change in the size of the silhouette area fron diastole to systole. We have however shown above from studies on cadavers that there is a tendency to underestimate volume from silhouette area when the heart is contracted so that to some extent the error due to diastole filling of the atria is offset by the error due to underestimation of volume from silhouette area.

The average output per beat in the sitting position was estimated as 37.8 per cent of the cardiac contents or 18.9 per cent from each ventricle. This corresponds closely with the 20 per cent estimate based on the work of Lindhard, as outlined above. The lowest output was 27.4 per cent of the cardiac content or 13.7 per cent from each ventricle. The largest was 58.2 per cent or 29.1 per cent from each ventricle. If we estimate the ventricular content as $33\frac{1}{3}$ per cent of the blood in the heart in the latter case the ventricle was nearly completely emptied at each contraction while in the former case it was less than half emptied. In eight out of the sixteen cases the per cent of cardiac blood expelled varied from 39.2 per cent to 41.8 per cent or close

TABLE 20

Volume of heart estimated from diastolic and systolic silhouette areas, difference in volume, percentage of reduction in heart volume and percentage of heart blood expelled during systole

SUBJECT	DIASTOLIC VOLUME	SYSTOLIC VOLUME	DIFFERENCE	PER CENT REDUCTION VOLUME	PER CENT HEART BLOOD EXPELLED	REMARKS
	cc.	*cc.*	*cc.*			
A. Sitting position						
J. F. S., 5′ 10″, 135 lbs.	691.2	595.8	95.4	13.8	27.4	
E. J. V., 5′ 6½″, 148 lbs.	861.8	741.0	120.8	140.3	7.8	Enlarged heart
E. F. S., 5′ 9″, 155 lbs.	723.9	619.2	104.7	14.46	28.7	
H. A., 5′ 9¾″, 152 lbs.	675.0	580.0	95.0	14.1	27.9	
	699.0	590.0	109.0	15.6	31.0	
J. C. G., 5′ 3″, 115lbs.	620.0	522.0	98.0	15.8	31.3	
R. W. T., 5′ 8¼″, 143 lbs.	667.4	555.6	111.8	16.75	33.3	
L. L. D., 6′ ½″, 169 lbs.	862.0	692.0	170.0	19.72	39.2	
C. C. V., 5′ 10″, 165 lbs.	732.9	587.8	145.1	19.8	39.3	
C. S. Z., 5′ 10″, 141 lbs.	612.0	490.0	122.0	19.93	39.5	
C. E. G., 5′ 11½″, 164 lbs.	775.0	619.0	156.0	20.13	40.0	
A. L., 5′ 4½″, 129 lbs.	657.8	522.0	135.8	20.64	41.0	
S. A. M., 5′ 8″, 171.5 lbs.	658.4	522.0	136.4	20.72	41.2	
P. M. D., 5′ 11½″, 146.3 lbs.	676.0	535.0	141.0	20.85	41.3	
F. C. K., 5′ 8″, 154 lbs.	750.0	586.0	164.0	21.87	41.8	Rather large heart
H. W. S., 5′ 7½″, 142 lbs.	699.6	514.0	185.6	26.25	56.1	
C. M., 5′ 10″, 145 lbs.	715.8	506.0	209.8	29.3	58.2	
Average	.....	.....	.....	19.04	37.8	
B. Prone position						
E. J. V., 5′ 6½″, 148 lbs.	887.0	792.3	94.7	10.68	19.2	
R. W. T., 5′ 8¼″, 143 lbs.	658.9	579.6	79.3	12.04	23.9	
W. E. G., 5′ 4½″, 130 lbs.	603.0	522.0	81.0	13.43	25.4	
J. F. S., 5′ 10″, 135 lbs.	741.9	635.2	106.7	14.38	28.5	
E. F. S., 5′ 9″, 155 lbs.	723.9	619.2	104.7	14.46	28.7	
H. A., 5′ 9¾″, 152 lbs.	699.0	590.0	109.0	15.60	31.0	
M. D. W., 5′ 9″, 137 lbs.	851.8	715.8	136.0	15.96	31.7	
C. E. G., 5′ 11½″, 164 lbs.	835.6	698.7	136.9	16.39	32.5	
Average	.....	.....	.....	14.1	27.6	

to the estimate given above of 20 per cent from each ventricle, 60 per cent of the ventricular content.

In the prone position we have observations on eight individuals. The average output was 27.6 per cent of the cardiac content or 13.8 per cent for each ventricle; 41.4 per cent of the ventricular content. The extremes are 19.2 per cent of the cardiac content, 9.6 per cent for each ventricle, 29 per cent of the ventricular content; and 32.5 per cent of the cardiac output, 16.8 per cent for each ventricle, or 50.4 per cent of the ventricular content. The estimates of percentage output of ventricular content are based upon the assumption that one-third of the blood in the heart in diastole is to be found in each ventricle, one-third in the two atria. It is probable however that in the prone position a greater proportion of the blood in the heart in diastole is to be found in the atria and that the percentage output from each ventricle is greater. On the assumption that in the prone position there is an equal amount of blood in each chamber of the heart in diastole the percentage output from each ventricle would average 55.2 per cent, with variations from 38.4 per cent to 62 per cent.

The relation of cardiac output as determined by the method given above, to various factors has been studied in our laboratories by Mr. E. J. Van Liere. He found no correlation between body weight, height, or build and the proportional amount of blood expelled at each contraction of the heart. Hearts whose diastolic volume was 5 per cent or more above the normal as compared with body weight showed less proportional cardiac output than normal and small hearts. High pulse pressure was accompanied by large relative output in a given position, although in the prone position the pulse pressure was higher than in the sitting position while the relative output was smaller. No definite correlation between systolic pressure or pulse rate and output was found under the conditions of the experiment.

The average left ventricular output sitting was 80.8 cc. per kilo per minute, the average pulse rate 82 making the output approximately 1 cc. per kilo per beat. The average output

lying was 56.9 cc. per kilo per minute with a pulse rate of 67 or 0.85 cc. per kilo per beat.

f. Relation of size of heart to height, age and sex

In the preceding sections we have considered heart size chiefly from the standpoint of body weight with which it is most closely correlated. In case of a given individual, however, other factors than merely body weight must be taken into consideration before we can form an accurate judgment as to whether or not the size of the heart is normal for that individual. Of these other factors the chief are height, age and sex.

The size of the heart for a given body weight is estimated in the tables on the assumption of normal height for that weight for a given sex and age. The chief studies on the relations of weight, height, sex and age in the adult have been made by the insurance actuaries. The most important of these studies is the Medico-Actuarial Mortality Investigation vol. 1 published by the Association of Life Insurance Medical Directors and the Actuarial Society of America. For the period of childhood and youth we have a large number of studies made on school children of which special mention may be made of those of Roberts ('78–'83), H. P. Bowditch ('75, '79, '91), F. Burk ('98), A. Key ('89), F. Boas ('96–'97), Hastings, W. W. ('02), Baldwin ('14), W. T. Porter ('94) and Ethel M. Elderton ('14–'15). The pioneer work in this general field is that of Quetelet ('32, '48).

These studies have shown that a closer correlation between height and weight is found if age and sex be taken into consideration than if these are ignored. The figures given in tables A and B are based upon an analysis of the data available in the literature together with studies made in the Clinical Department at the University of Wisconsin. A full account of these studies is reserved for publication in a subsequent paper. The figures for height are those which these studies have led us to believe represent a fair normal average for a given weight, for the age and sex indicated in healthy Americans. Weight means weight without clothes; height, height without shoes; age, age at nearest birthday.

Table A gives figures for childhood and youth. Table B gives figures for adults at three ages, 20, 30, and 50. In making use of these tables one compares the parellel ray silhouette area of the heart of the individual under consideration (a) with the silhouette area given by the table as normal for a person of the individual's weight and (b) with the silhouette area normal for a person of the individual's height, sex and age being taken into consideration. If the silhouette area is normal for weight or for height or is intermediate between the two we consider that the heart is one of normal size. If the heart volume corresponding to the silhouette area is more than 10 per cent too large both from the standpoint of height and of weight we consider that it is disproportionately large. If it is correspondingly small both from the standpoint of height and of weight we consider it disproportionately small. Our own practice is thus to estimate cardiac size in percentage of variation of volume from that assumed as normal for height and from that assumed as normal for weight. For instance we will suppose that a man 30 years of age 5′ 10″ tall and weighing 150 pounds shows a heart silhouette area (reduced about 6 per cent to allow for divergence of rays if a radiograph is used) of 120 sq. cm. From table B we find that an area of 120 sq. cm. corresponds to a volume of 696 cu. cm. For a weight of 150 lbs. we should expect a volume of 723 cu. cm. In a man 5′ 10″ tall at 30 years of age we should expect a volume of 768 cu. cm. The heart of the individual under consideration is therefore 27 cu. cm. or 3.7 per cent below the standard from the standpoint of weight, 72 cu. cm. or 9.1 per cent below the standard for height. A slight variation of this kind is within the limits of error of the method used and the heart would be considered of normal size.

In conclusion I desire to thank the members of the staffs of the departments of anatomy, physiology and clinical medicine and Prof. Max. Mason of the department of physics for valuable aid in carrying out the investigations described in this paper.

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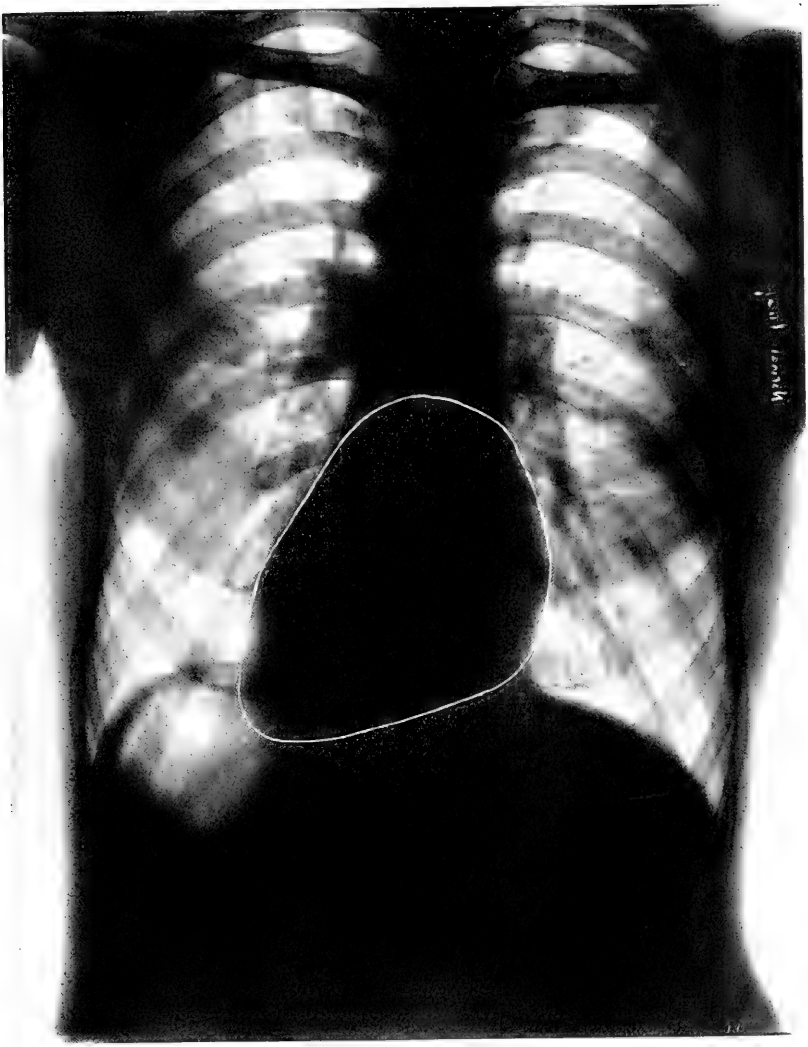
PLATE 1

EXPLANATION OF FIGURE

Radiograph of the heart of a man, height 5′ 11$\frac{3}{8}$″, weight 146.3 lbs., age 43. The right and left margins of the heart which are clear in the photograph have been outlined with plain lines of white ink. The upper and lower borders, which have been arbitrarily completed, are likewise shown by lines of white ink. The silhouette area is 127.5 sq. cm. Reduced 6 per cent gives a net area of 119.9 sq. cm. The transverse diameter is 12.5 mm. Reduced 3 per cent gives 12.1 mm. as the net transverse diameter. Comparisons of the measurements of this heart with the normal standard are as follows:

	WEIGHT	HEIGHT	AGE	TRANSVERSE DIAMETER	AREA	VOLUME
	pounds				*sq. cm.*	*cc.*
Observed	146.3	5′ 11$\frac{3}{8}$″	43	12.1	119.9	
Standard for weight	146.3	5′ 5″	43	13.0	120.5	700
Standard for height and age	179.0	5′ 11$\frac{3}{8}$″	43	13.9	138.0	860
Standard for area	144.6	5′ 5″	43	12.9	119.8	694

The heart is close to the standard size from the standpoint of weight but is small (vol. -166 cc., −19.3 per cent) from the standpoint of height. The individual is decidedly thin from the standpoint of height and age. The width of the silhouette is narrow compared with the area as we should expect in an individual of this build.



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